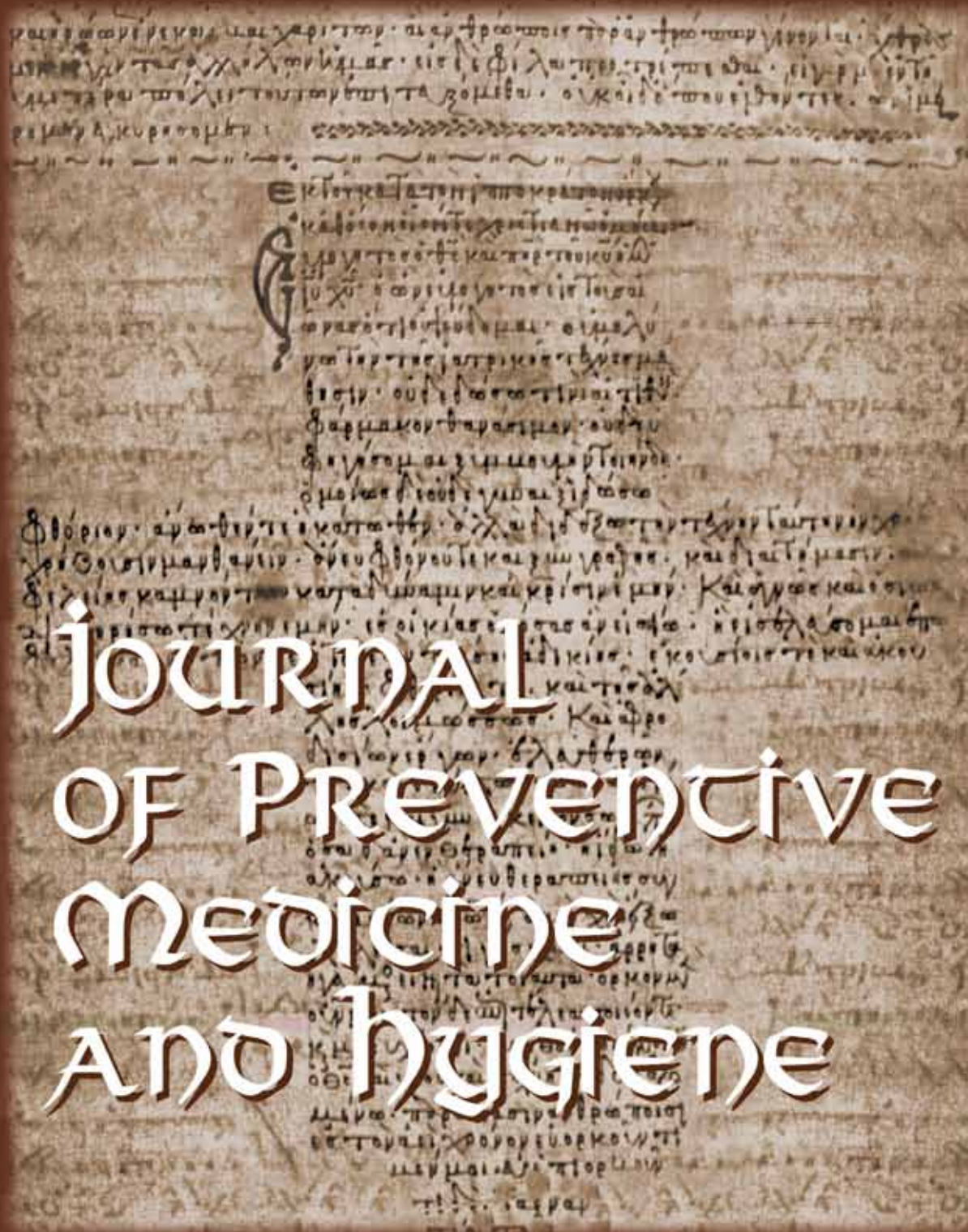


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CONTENTS

Original articles

- Adolescents: which risks for their life and health?
E. Balocchi, G. Chiamenti, A. Lamborghini 191
- Stress among medical Saudi students at College of Medicine, King Faisal University
A.G. Abdel Rahman, B.N. Al Hashim, N.K. Al Hiji, Z. Al-Abbad 195
- Prevalence survey of healthcare-associated infections and antimicrobial use at the University Hospital
“Paolo Giaccone”, Palermo, Italy
I. Sinatra, L. Carubia, V. Marchese, L. Aprea, N. D'Alessandro, C. Mammina, M.V. Torregrossa 200
- Staphylococcus aureus* infections in children in an Iranian referral pediatric Hospital
F. Sabouni, R. Ranjbari, B. Pourakbari, S. Mahmoudi, M. Teymuri, M. Taghi Haghi Ashtiani, Z. Movahedi, S. Mamishi 205
- Social and interpersonal relationship modifications after renal transplant. A statistic and epidemiologic evaluation
F. Famà, E. Micali, C. Linard, M.D. Venuti, G. Costantino, M. Giofrè-Florio 208
- A study on occupational exposure of Sicilian farmers to *Giardia* and *Cryptosporidium*
F. Di Piazza, M.A. Di Benedetto, C.M. Maida, S. Glorioso, G. Adamo, T. Mazzola, A. Firenze 212
- Psychosocial predictors of barriers to cervical cancer screening among Iranian women:
the role of attachment style and social demographic factors
K. Hajializadeh, H. Ahadi, F. Jomehri, M. Rahgozar 218
- Antimicrobial potential of Sicilian honeys against commensal *Escherichia coli* and pathogenic *Salmonella* serovar
Infantis
M.A. Coniglio, G. Faro, G. Giammanco, S. Pignato, M. Marranzano 223

ORIGINAL ARTICLE

Adolescents: which risks for their life and health?

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Key words

Adolescent • Harm reduction • Risk taking • Young adult

Summary

This paper reviews emerging research findings related to the biological, behavioral, psychological and social processes that occur during adolescence. New research makes it possible to identify interactions among brain structures, hormonal production and

behavioral impulses, that underpin and explain the connections with serious problems emerging during adolescence: behavioral disorders, substance abuse, risky sexual conduct, violence and other social disorders.

Adolescence is defined as the period of life that starts with the biological, hormonal and physical changes of puberty and ends at the age at which an individual attains a stable, independent role in society. Adolescents' health and well-being is threatened by their inclination to engage in risky and reckless behavior. Factors affecting adolescence, including rapid developmental changes and physical growth, family and social contexts, mean that risk behavior at this stage is different in significant ways from adult behaviour [1]. Adolescents engage in more risky behavior than adults, although the magnitude of age differences in risk-taking varies; as a general rule, adolescents and young adults are more likely than adults to binge drink, smoke cigarettes, have casual sex partners, engage in violent and other criminal behavior, and have fatal or serious accidents.

Significant progress has occurred in the study of adolescent risk-taking, integrating findings across disciplines as neuroscience, psychology, sociology. The last decade has been one of sustained interests and technological advances that have created a lot of opportunities for researchers to learn more about the brain. Longitudinal MRI studies have revealed that a second surge of neuron growth peaks in the brain just before puberty [2, 3].

We know that changes that occur during adolescence are functionally linked to changes in brain structure. Adolescence is a period of "brain plasticity", when extraordinary brain changeability occurs [4].

Plasticity, the brain's ability to change in response to repeated stimulation, is important during adolescence because allows us to learn and adapt; it consists in an elaboration and stabilization of a synaptic architecture constructed as part of the process of learning, but also increases the vulnerability of adolescents toward making unhealthy decisions. Their brain circuitry is still being formed: re-wiring, neuron proliferation and pruning thus make it difficult to think critically before making choices. Neural connections can also be forged and re-fined or weakened and discarded during plasticity [2].

It was widely assumed that the vast majority of brain development takes place in the first few years of life, but the brain develops right throughout adolescence into the twenties and thirties [5]. This important discovery helped to increase understanding about adolescent brain development and provided support for many unexplained behaviors experienced in late adolescence and early adulthood.

This surge, exactly like the one that occurs during infancy, consists of a thickening of the brain's gray matter. Neurons grow and increase their ability to connect with other neurons. Following this period of proliferation, the brain "rewires" itself over the course of adolescence, especially in the prefrontal cortex. The rewiring is accomplished by two mechanisms: myelination (a process increasing the speed of conduction of nerve impulses) and neural pruning (a process to clean out the unused synapses).

Over the course of adolescence, connections between different areas and structures of the brain increase and strengthen, allowing for multi-tasking, better ability to solve problems and greater capacity to process increasingly complex information.

The processes of neuron proliferation and pruning occurring in the adolescent years result in a brain that is very malleable and adaptable in the face of challenges posed in the environment and highly subject to molding by experience. Experience and use the circuits of the brain are shaped through practice. This is how the brain consolidates learning. This implies that to some extent adolescents can influence the wiring of their own brains by the activities they engage in, the experiences they seek and the things they spend their time doing. Trauma, chronic stress, substance use, and even sedentary activities may have greater negative effects when experienced during this sensitive period of brain development.

Advances in brain research have shown that hormones are not the only explanation for erratic adolescent behaviour [6]. Researchers are still trying to establish the

exact nature of the interrelationships between pubertal processes and adolescent brain development. The timing of brain changes may underlie many aspects of adolescent behavior and risk taking. As for the brain changes that precede puberty, the brain itself initiates puberty by releasing hormones. Brain changes related to pubertal hormones appear to occur mainly in the structures of the brain most closely linked with emotion, arousal and motivation, and with appetite and sleep patterns (Limbic System). Brain changes independent of puberty, as indicated by adolescent brain research, are those related to the development of advanced cognitive functioning (Prefrontal Cortex). Pubertal-related hormones directly influence physical drives (such as appetite and sleep) motivations and emotions, whereas cognitive and regulative controls over behaviour are established in a more gradual and indirect manner.

The limbic system, sometimes referred to as the “emotional brain,” is involved in the expression of emotions (fear, anger) and motivation, particularly those related to survival. The limbic system is also involved in feelings of pleasure that reward behaviors related to species survival, such as eating and sex. In addition, limbic system structures have functions related to memory storage and retrieval, particularly memories related to events that invoked a strong emotional response.

Different studies [7] involving brain scans revealed that when interacting with others, and making decisions, adolescents are more likely than adults to be influenced by their emotions. In addition, adolescents often “read” others’ emotions incorrectly. Studies involved comparing a teen brain to an adult brain determined that adolescents’ prefrontal cortex (where “logical” thinking occurs) is used less often during interpersonal interactions and decision making than their adult counterparts’. Instead, teens relied more on the emotional region of their brains when reading other’s emotions, which is more impulsive [8].

An understanding of what is going on in the limbic system during adolescence helps provide a partial explanation for some characteristic and familiar adolescent behaviors such as: quickness to anger, intense mood swings and making decisions on the basis of feelings. Adolescents’ brains are still rooted in the “emotional brain” making it challenging to make what adults consider to be logical and appropriate decisions.

Brain-wise, the most dramatic happens to the prefrontal cortex, the area of the brain involved in high level cognitive functions such as decision-making, planning, social interaction and self-awareness. MRI studies show that this region undergoes quite dramatic change during adolescence. In fact, there’s a significant decline in the prefrontal cortex in adolescence because the brain is finetuning itself.

As adolescents progress toward adulthood, the self-regulatory parts of their brains are still evolving. An earlier onset of puberty increases the vulnerability for today’s teens, making them more susceptible to take risks that effect their health and development over a longer period of time. Self-regulation is broadly described among psy-

chologists as the management of emotions and motivation. It also involves directing and controlling behavior to meet the challenges of the environment and to work toward a conscious purpose. Self-regulation also encompasses affect regulation, which entails controlling the expression of intense emotions, impulse control, and delaying gratification.

Such behavioral control requires a higher level of cognitive and executive functions. These functions reside in the prefrontal cortex, which matures independent of puberty and is still evolving and developing well into an individual’s mid twenties. During this period of development, adolescents should not be over-protected, but allowed to make mistakes and learn from their experiences and practice self-regulation.

The prefrontal cortex is responsible for cognitive analysis and abstract thought, and the moderation of “correct” behavior in social situations. The prefrontal cortex takes in information from all of the senses and programs thoughts and actions to achieve specific goals [9].

The prefrontal cortex is one of the last regions of the brain to reach maturation. This delay may help to explain why some adolescents act the way they do. This region is involved in functions like attention, problem solving, evaluating consequences of behaviour, making predictions, planning, balancing short-term rewards with long term goals, impulse control and delaying gratification, emotions.

This brain region gives an individual the capacity to exercise “good judgment” when presented with difficult life situations

MRI studies of the brain show that developmental processes tend to occur in the brain in a back to front pattern, explaining why the prefrontal cortex develops last. These studies indicate that brain development is not complete until near the age of 25, referring specifically to the development of the prefrontal cortex.

The better flow of informations between brain regions is allowed by a process of myelinisation of connections [10]. This body of brain research data has led to the idea of a progressive growth of the prefrontal cortex that gradually becomes able to oversee and regulate the behavioral responses initiated by the more primitive limbic structures [11]. The brain area is more active in adolescents when they make decisions than it is in adults – which suggests that adolescents really do use a different mental approach to make social decisions. The ability to account for someone else’s perspective is still developing even in relatively late-stage adolescence.

During adolescence, white matter increases in the corpus callosum, the bundle of nerve fibers connecting the right and left hemispheres of the brain. This allows for enhanced communication between the hemispheres and enables a full array of analytic and creative strategies to be brought to respond to the complex problems that may arise in a young person’s life. The role of experience is critical in developing the neural connectivity that allows for conscious cognitive control of the emotions and passions of adolescence. Teens who take risks in relatively safe situations exercise the circuitry and de-

velop the skills to control themselves in more dangerous situations.

With an immature prefrontal cortex, even if teens understand that something is dangerous, they may still go ahead and engage in the risky behavior. Recognizing the asynchrony of development of the regions of the brain helps us to see adolescent risk-taking in a new light [12]. The most prevalent risks adolescents are sexual risk-taking, substance use, injury and violence being the leading causes of death in age 10-24 years of age, (motor vehicle crashes: 30%; homicides: 15%, and suicide: 12%). Alcohol and drug use have a key-role in approximately 41% of deaths related to motor vehicle crashes. Risky sexual behaviors are the cause of almost half of the 19 million new sexually transmitted infections diagnosed each year in the U.S. adolescents 15-24 years old [13]. Adolescents take risks to test and define themselves. Especially among males, risk-taking makes good evolutionary sense: it can lead to situations where new skills are learned and new experiences can prepare them for future challenges. Risk-taking serves as a mean for discovery about oneself, others and the larger world. The natural and normative proclivity for risk-taking plays a central role in adolescent development, making it a time of both great potential and great vulnerability.

It is important to understand how the changes that occur in the brain influence adolescent risk-taking behaviour. Developmental psychologists have measured and documented a jump in cognitive capabilities in early adolescence. Beginning around the age of 12, adolescents decrease their reliance on concrete thinking and begin to show the capacity for abstract thinking, visualization of potential outcomes, and understanding of cause and effect. Teens begin looking at situations and deciding whether it is safe, risky, or dangerous [14].

These aspects of development correlate with the maturation of the frontal lobe, a shift from expanding neural connections to pruning and an increase in hormones released; all of which drive an adolescent's mood and impulsive behavior. By age 15, studies show there is little difference in decision-making about hypothetical situations between adults and adolescents [14].

Teens were found capable of reasoning about the possible harm or benefits of different courses of action. However, in the real world, adolescents still engaged in dangerous behaviors, despite understanding the risks involved. Both the role of emotions and the connection between feeling and thinking need to be considered: thinking under conditions of intense emotions and high arousal leads teens to make poorer decisions. When circumstances are less intense teens can make better decisions. With the addition of all the complex feelings, such as fear of rejection, wanting to look "smart", the excitement of the risk, or anxiety, it is even more difficult for teens to think through potential outcomes, understand consequences of their decisions, or even use common sense.

The immaturity of the connections between the limbic system and the prefrontal cortex, and the research around the amygdala, provide support to this theory. The

nucleus accumbens, a part of the brain's reward system located in the limbic system, is the area that processes information related to motivation and reward. Brain imaging studies have shown that the nucleus accumbens is highly sensitized in adolescents, sending out strong impulses to act when faced with the opportunity to obtain something desirable [15]. These regions have been implicated in diverse aspects of social processing, including the recognition of socially relevant stimuli (expressions, social judgments, appraisal of others, judging attractiveness, assessing other's intentions, social reasoning, and many other aspects of social processing). Interestingly, among adolescents the regions that are activated during exposure to social stimuli overlap considerably with regions also shown to be sensitive to variations in reward magnitude. Because these same regions have been implicated in many processes of reward-related affect it is suggested that, at least in adolescence, social acceptance by peers may be processed in ways similar to other sorts of rewards, including nonsocial rewards [16].

When teens are alone they make safer decisions, but in the presence of friends the teens make riskier decisions. This helps to explain why so much adolescent risk-taking occurs in the context of the peer group. Peer influence is an important component of adolescence development and drives to both positive and negative outcomes [12]. In adolescence, teens begin to develop friendships that are more intimate and longer lasting and at the same time have multiple groups of friends that are similar in demographics or interests. These friendships allow teens to develop their identities and define themselves independently of their parents, feel accepted, and practice social skills.

Healthy friendships provide support for challenges teens encounter in adolescence and provide positive experiences during these years of intense change. However, when teens find themselves in emotionally arousing situations and with their immature prefrontal cortex, cognition thinking may be impaired, and they may be more likely to take reckless actions and make impulsive decisions [12].

Mass media, community norms, and adult role models also influence adolescent risk-taking behaviors. Teens are constantly bombarded with emotionally arousing, and often dangerous messages through multi-media: unprotected sex, substance abuse, and alcohol use, which even adults have trouble resisting to.

During puberty, the increases in estrogen and testosterone bind receptors in the limbic system, which not only stimulates their sex drive, but also increases teens' emotional impulsivity. Changes in the brain's reward sensitivity that occur during puberty have also been discovered. These are related to changes in dopaminergic neurons, circuits that produce feelings of pleasure (among other functions). The increase in risk-taking between childhood and adolescence is due primarily to increases in sensation seeking that are linked to changes in patterns of dopaminergic activity around the time of puberty [17].

Due to these changes, teens may require higher levels of stimulation to achieve the same levels of pleasure or reward, leading them to seek out new experiences, and driving them to make riskier decisions. The early reorganization of dopaminergic neurons in the motivational system, due to the secretion of sex hormones (mostly estrogens, testosterone and oxytocin) at the beginning of puberty, impels adolescents toward thrill seeking. On the other hand, the slow maturation of the cognitive control system, mostly exerted by the prefrontal cortex, implies that these impulses cannot be appropriately regulated [18].

“Adolescents are more vulnerable than any other age group to developing nicotine, alcohol, and other drug additions because of the regions of the brain that govern impulse and motivation are not yet fully formed” [19].

Parents can help adolescents through this period by listening, offering support, and guidance.

Teens need to be surrounded by caring adults and institutions that help them learn specific skills and appropriate adult behavior. Suggestions should answer more challenging and specific questions about specific systems and practices, that will best help them grow and mature in appropriate ways and opportunities that will be most effective in helping them develop the skills of judgment, planning and impulse control.

Having knowledge of adolescent brain development can help to understand why teens take risks and that risk-taking behavior is a normal and necessary part of adolescence. This knowledge can assist in developing effective interventions that focus on reducing the harm associated with risk-taking behaviour, to establish a safer, healthier, more teen friendly environment.

References

- [1] Taylor SJ, Barker LA, Heavey L, et al. *The typical developmental trajectory of social and executive functions in late adolescence and early adulthood*. Dev Psychol 2013;49:1253-65.
- [2] Giedd JN, Blumenthal J, Jeffries NO, et al. *Brain development during childhood and adolescence: a longitudinal MRI study*. Nature Neuroscience 1999;2:861-3.
- [3] Dosenbach NUF, Nardos B, Cohen AL, et al. *Prediction of individual Brain Maturity Using fMRI*. Science 2010;329:1358.
- [4] Lebel C, Beaulieu C. *Longitudinal development of human brain wiring continues from childhood into adulthood*. J Neurosci 2011;31:10937-47.
- [5] Stiles J, Jernigan TL. *The basics of brain development*. Neuropsychol Rev 2010;20:327-34.
- [6] Sisk CL, Foster DL. *The neural basis of puberty and adolescence*. Nature Neuroscience 2004;7:1040-478.
- [7] Guyer AE, McClure-Tone EB, Shiffrin ND, et al. *Probing the neural correlates of anticipated peer evaluation in adolescence*. Child Dev 2009;80:1000-15.
- [8] Cohen Kadosh K, Johnson MH, Dick F, et al. *Effects of age, task performance, and structural brain development on face processing*. Cerebral Cortex 2013;23:1630-42.
- [9] Casey BJ, Jones RM, Hare TA. *The adolescent brain*. Ann NY Acad Sci 2008;1124:111-26.
- [10] Giedd JN. *Structural magnetic resonance imaging of the adolescent brain*. Ann NY Acad Sci 2004;1021:77-85.
- [11] Steinberg L. *A social neuroscience perspective on adolescent risk-taking*. Dev Rev 2008;28:78-106.
- [12] Steinberg L. *Risk taking in adolescence: what changes and why?* Ann N Y Acad Sci 2004;1021:51-8.
- [13] <http://www.cdc.gov/mmwr/pdf/ss/ss6104.pdf>- Accessed March 2013
- [14] Steinberg L. *Cognitive and affective development in adolescence*. Trends Cogn Sci 2005;9:69-76.
- [15] Gardner M, Steinberg L. *Peer Influence on risk taking, risk preference and risky decision-making in adolescence and adulthood*. Dev Psych 2005;41:625-35.
- [16] Van Leijenhorst L, Zanolie K, Van Meel CS, et al. *What motivates the adolescent? Brain regions mediating reward sensitivity across adolescence*. Cereb Cortex 2010;20:61-9.
- [17] Lopez, Schwartz SJ, Prado G, et al. *Adolescent neurological development and its implications for adolescent substance abuse prevention*. J Prim Prev 2008;29:5-35.
- [18] Barbalat G, Domenech P, Vernet M, et al. *Risk-taking in adolescence: A neuroeconomics approach*. Encephale 2010;36:147-54.
- [19] Carls J. *New research on adolescent brain development*. Center for Substance Abuse Prevention. 2004 November. Issue #1.

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ORIGINAL ARTICLE

Stress among medical Saudi students at College of Medicine, King Faisal University

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Key words

Stress • medical students • Risk factors of stress • Social risk factors • Psychological risk factors

Summary

Background. Sources of student stress can be academic pressures, social or personal issues and medical students have to face the challenge of rigorous curriculum and also have to learn how to deal with emotionally difficult experiences.

Aim of Work. Determination of the prevalence of stress and to identify the risk factors of stress among Saudi medical students at Faculty of Medicine, King Faisal University, Saudi Arabia.

Methods. A survey study design was conducted among medical students at Faculty of Medicine, King Faisal University, Al Ahsaa Governorate, Eastern Province from February-May, 2013. Both male and female students were invited to participate in our study. The total number of students was 650 Saudi medical students, out of them 244 medical students were participated in the current study. All participants were provided a self administered questionnaire. A likert scale with 3 points for responses was used. Questionnaires were given to participants 2 month before end-semester examinations, to minimize the extra stress symptoms A score of stress was calculated.

Results. Prevalence of stress was 53% among Saudi students. Gender has no role in stress among medical students ($p > 0.05$). Logistic regression analysis revealed the most important risk factors of stress, having places for recreation at the University showed a relationship with stress among medical students with a p value = 0.000, there is a statistically significant relationship between stress and having optimal place for studying outside the university $p = 0.001$, while, comparing number of sleeping hours with stress, we could not find a statistically significant relationship among medical students, $p = 0.744$. Medical students who had close friends to share with them their stresses and concerns showed a highly statistically significant relationship between stressed and unstressed students $p = 0.001$.

Conclusion. Medical students reported high levels of stress. The most frequently occurring stressors among the students were related to academic and psychosocial domains. The associations between stressed cases and gender, occurrence of academic and psychosocial stressors need to be further tested by prospective studies.

Introduction

University student's life is subject to many different kinds of stress, and sources of student stress can be academic pressures, social or personal issues and medical students have to face the challenge of rigorous curriculum and also have to learn how to deal with emotionally difficult experiences [1]. It is usually observed that medical students undergo tremendous stress during various stages of the medical education. Stress is defined as the body's non-specific response to demands made upon it, or to disturbing events in the environment. It is not just a stimulus or a response but rather, it is a process by which we perceive and cope with environmental threats and challenges. Personal and environmental events that cause stress are referred to as stressors [2-4]. In addition to coping with the normal stressors of everyday life, medical sciences students must deal with stressors specific to medical school, which include information and input overload, financial indebtedness, lack of leisure time, and pressures of work, work relationships and career choices [5]. Recently stress during medical training is increasingly being reported in published literature.

Previous studies have shown fairly high levels of distress, such as symptoms of depression [6, 7] and even suicide thoughts among medical undergraduates [8, 9]. The potential negative effects of emotional distress on medical students include impairment of functioning in class-room performance and clinical practice, stress-induced disorders and deteriorating performance [10, 11]. Sources of student stress can be academic pressures, social or personal issues, and financial problems. In recent years, there has been a growing appreciation of the stresses involved in the training of health professionals [1]. Medical programs have always been regarded as a popular choice for tertiary education. Only those who have excellent academic achievement can be successful in the course. Therefore, the medical program may be even more competitive and stressful for students who are accepted [12, 2]. In addition to stress the students' social, emotional and physical as well as family problems may influence their learning ability and academic performance [13]. Students often begin their medical education unaware or emotionally unprepared to cope with these challenges. There is increased interest among medical educators and administrators to promote a cul-

ture of wellness, professionalism, self-care, and help-seeking in medical students. In the current study we aim to determine the prevalence of stress and to determine the risk factors of stress among Saudi medical students at Faculty of Medicine, King Faisal University, Saudi Arabia.

Subjects and methods

A survey study was conducted among Saudi medical students (preparatory, first year, 3rd year, 5th year and 6th year medical students) at Faculty of Medicine, King Faisal University, Al Ahsaa Governorate, Eastern Province from February-May, 2013. All male and female students in these years were invited to participate in our study. The total number of students is 650 medical students, out of them 244 medical students were participated in the current study. All participants were provided a self administered questionnaire. Consent of the Faculty of Medicine administrative was provided before delivery of questionnaires. A written consent with assurance of confidentiality was provided from all participants. A self administered questionnaire of 4 sections was constructed in order to determine the factors that may cause stress among medical students Faculty of Medicine, KFU. The 5 sections included personal data (age, sex, marital status and year of study), factors related to the content of the curriculum and method of learning and exams, familial factors, health status factors and social, a separate section for stress score risk factors. The self administered questionnaire was constructed in Arabic and back translated into English version. A pilot testing of the questionnaire was done before data collection for validity of the questionnaire. Modifications were performed according to pilot study results. A likert scale with 3 points for responses was used. Questionnaires were given to participants 2 month before end-semester examinations, to minimize the extra stress symptoms. Data collection was performed in two weeks. The questionnaires were distributed to the participants during face-to-face sessions inside the lecture halls separately according to the year of study. The process of filling in the questionnaires took one day to complete and they were to be returned on the next day. A score for stress was calculated using the mean score for all risk factors related to the stress section in the questionnaire and divided into stressed and not stressed. The mean score of all risk factors was calculated. All participants below the mean score were considered as not stressed students and all participants above the mean score were considered as stressed students.

Data analysis

All data collection forms were given serial numbers. Data were entered, checked for data entry errors, explored and cleaned. Data were interpreted using an alpha (α) set at 0.05 with two tailed direction and confidence in-

terval of 95%. Descriptive analysis was done in the form of frequency, percent, mean and standard deviation and graphs. Initial comparisons were done using Chi-Square test for categorical variables, and student-t-test for quantitative variables. The risk factors for stress among medical students were obtained using the logistic regression analysis. The dependent variable was the presence and absence of stress in all the students. All variables described previously were considered as possible candidates for the final model. The initial multivariable model construction consisted in the preliminary selection of variables using a manual, purposeful selection method and a relatively large significance level (a approximately 0.25). Subsequently, the resulting model was reduced using a likelihood ratio test with a significance level of 0.05. The calibration of the final model was assessed using the Hosmer and Lemeshow goodness-of-fit test, and its discrimination was assessed by the area under the receiver operator characteristics curve. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 17.0 (SPSS Inc. Headquarters, Chicago, Illinois, USA).

Results

The total number of the medical students participated in the current study was 244, both genders male and female students were participated. Students were distributed as 29% from preparatory year, 42% first year, 26% from third year, and only 3% from sixth year. The mean of their age 20.3 ± 1.7 , the range was (18-25). The mean age of stressed students was 19.9 ± 1.4 compared with mean age of unstressed students of 20.7 ± 1.9 with a p value of 0.001. Table I showed the distribution of the demographic characteristics among the Saudi medical students at Faculty of Medicine. Prevalence of stress is 53% among medical Saudi students. Gender has no role in stress among medical students ($p > 0.05$). Table II revealed the factors that are related to the content of curriculum, method of learning and exams that cause stress among medical Saudi students at Faculty of Medicine. Table III showed the distribution of stress among the Saudi medical students according to familial factors that may lead to stress among students, all factors showed highly statistical significance between stressed and unstressed students. Table IV showed the mixture of all risk factors in causing stress among medical students by using regression analysis. Having places for recreation at the University showed a relationship with stress among medical students with a p value = 0.001, additionally, there is a statistically significant relationship between stress and having optimal place for studying outside the university $p = 0.001$, while, comparing number of sleeping hours with stress, we could not find a statistical significance relationship among medical students, $p = 0.744$. Medical students who have close friends to tell them about their stresses and concerns showed a highly statistical significance between stressed and unstressed students $p = 0.001$

Tab. I. Distribution of the demographic characteristics among the Saudi medical students at Faculty of Medicine, King Faisal University.

	Number = 244	Percent
Age of students Mean $\pm$ SD Range	20.3 $\pm$ 1.7 (18-25)	
Gender of students Male	142	(58.2%) (41.8%)
Marital status of students Single	184	(75.4%)
Residence Al Hassa	206	(84.4%)

When percentages of 2 groups are reported it is not necessary to report them for both groups (if females are 30% it is obvious that males are 70%).

In Table I an average age of 15 years is reported, that is not possible for medical students.

Some results are reported in Tables, some others in Figures with different formats. The results of a unique logistic regression must be reported in a Table with the same format for all the included variables.

The OR reported in Table 4 are not understandable: which is the measurement scale? What does an OR = 500 mean? Which is the direction of the association?

Do not write $p = 0.000$ but rather $p < 0.001$.

Tab. II. Stress among Saudi medical students due to methodology of studying, learning and exams at Faculty of Medicine, King Faisal University.

	Stress N=129 (%)	No stress N=115 (%)	P value
Finishing all curriculum before entering exam Yes No	44 (63.8%) 85 (48.6%)	25 (36.2%) 90 (51.4%)	0.032
Questions for exams are suitable for my abilities and suitable for curriculum in its number and difficulty Yes No	24 (57.1%) 105 (51.9%)	18 (42.9%) 97 (48.1%)	0.562
Number of exams is suitable for me to be motivated to study all the time Yes No	67 (44.1%) 62 (67.4%)	85 (55.9%) 30 (32.6%)	0.001
I have plan to study and I use it to organize my time during my studies Yes No	106 (59.6%) 23 (34.8%)	72 (40.4%) 43 (65.2%)	0.001
I have some difficulty in understanding the information in textbooks and it waste my studying time Yes No	117 (55.9%) 12 (34.3%)	92 (44.1%) 23 (65.7%)	0.017

Tab. III. Stress due to familial risk factors among Saudi medical students at Faculty of Medicine, King Faisal University.

	Stress N=129 %	No stress N=115 %	P value
Living with your parents Yes No	113 (57.9%) 16 (32.7%)	82 (42.1%) 33 (67.3%)	0.002
Motivation of the family for your study Yes No	112 (59.3%) 17 (30.9%)	77 (40.7%) 38 (69.1%)	0.001
Acceptance of the family for your study and your future career Yes No	114 (56.4%) 15 (35.7%)	88 (43.6%) 27 (64.3%)	0.014
Parents expectancy that you will get high marks make you nervous Yes No	103 (58.2%) 26 (38.8%)	74 (41.8%) 41 (61.2%)	0.007

Tab. IV. Factors associated with stress among medical students in the multivariable analysis.

	Odds Ratio	95% Confidence Interval	P value
Living with parents	0.48	0.128-1.814	0.281
Failing in previous exams	0.40	0.134-1.209	0.105
Concentrating in studying before exams	0.489	0.210-1.137	0.097
Finishing all curriculum before entering exams	0.481	0.195-1.186	0.112
Number of exams is suitable for students to motivate them to study all the time	0.351	0.156-790	0.011
There are places for amusement in the university students can use	0.303	0.131-0.699	0.005
Having optimal place for studying	0.355	0.163-774	0.009
Students' Family motivation to study	0.544	0.217-1.365	0.195
Acceptance of families to the career of study	0.331	0.110-0.992	0.048
Thinking in health status	0.263	0.111-0.624	0.002
Planning and organizing time of study	0.365	0.152-0.873	0.023
Family expectations of getting high marks	0.394	0.170-0.912	0.030
Having difficulty in understanding information in textbooks	0.579	0.192-1.749	0.333
Doing great efforts in studying	2.562	1.058-6.203	0.037
Age of students	1.259	0.953-1.664	0.105
Gender of students	1.774	0.819-3.841	0.146

Discussion

Stress is a physical, mental, or emotional response to events that causes bodily or mental tension. In small amounts, stress is normal and can help us be more active and productive. However, very high levels of stress experienced over a prolonged period can cause significant mental and physical problems [1]. In our study, we evaluated perceived stress among medical students including its sources such as psychosocial, academic performance, familial factors which may be of great importance to students as well as their teachers to improve their academic achievement. In the current study the prevalence of stress among medical students is 54.8%, in studies done by Sherina et al; (2003) and Zaid and Chan (2007) prevalence of stress among medical students ranged from 30% to 50% which reveal that our study have a higher prevalence of stress among medical students [12, 13]. Additionally, a prevalence of 41.9% and 46.2% were measured in a Malaysian government medical school and in a Malaysian private medical school, which were less than our prevalence of stress in the current study [3]. Also, the current study showed that there is a highly statistical significance between year of study and stress, preparatory year and first year of medical studies are the two major years that compose the stress and then stress decreased with progress of study to the sixth year. One of the explanations for the increase stress among younger students could be that medical program may be even more competitive and stressful for students who are accepted [2]. Stress among medical students was perceived in our study by regression analysis, exam questions and all related curriculum activities showed a highly statistical significant difference between stressed and unstressed students. Shah et al; (2010) found that there was no statistical significant correlation between perceived stress and academic per-

formance compared to our study [14]. This might be among the students who are striving to perform well in examinations may make themselves stressed. Among academic stressors, tests/exams were the chief sources of stress. Those students who perceive tests/ exams as a burden may experience stressful situations, while for others, who consider exams useful and help them in their learning. In our study recreational facilities was revealed as one of the prominent factors of stress among medical students with a highly statistical significance difference among stressed and unstressed medical students. Redwood and Pollak (2007) found that psychosocial factors such as insufficient recreational facilities were an important source of stress [15]. In regression analysis there is a high Odds ratio of psychosocial and familial factors among our students in the current study, which might suggest a linear relationship between these factors and stress which reflect that students who have academic stress factors can have also psychosocial and familial factors. Overall prevalence of stress in this study was 53.3% in male student and 46.7% in female student. Our study concluded that male students have more stress than female. The stress caused undesirable effects on their general health. This might be because male students need to finish study with high scores and at optimum time to start their careers. In conclusion, medical students were more frequent victims of academic stress than other students. It was possibly due to their higher academic demands and perception of time constraints to fulfill them. Important causes of stress among medical students included unsuitable teaching methods; an unsatisfactory college study environment; fear of failure in examinations, and social problems all of which resulted in perceived anxiety and depression, negative life-style practices and a worse status of physical and mental health changes since the start of their college studies [14, 16, 17].

References

- [1] Gomathi KG, Ahmed S, Sreedharan J. *Psychological health of first-year health professional students in a Medical University in the United Arab Emirates*. Sultan Qaboos Univ Med J 2012;12:206-13.
- [2] Yusoff MSB, Abdul Rahim AF, Yaacob MJ. *Prevalence and sources of stress among University Sains Malaysia medical students*. Malays J Med Sci 2010;17:30-7.
- [3] Aktekin M, Karaman T, Senol YY, et al. *Anxiety, depression and stressful life events among medical students: a prospective study in Antalya, Turkey*. Med Educ 2001;35:12-7.
- [4] Myers DG. *Stress and health*. In: *Exploring Psychology* (6th ed.). New York: Worth Publishers 2005, p. 402.
- [5] Sharifirad G, Marjani A, Abdolrahman CH et al. *Stress among Isfahan medical sciences students*. J Res Med Sci 2012;17:402-6.
- [6] Dyrbye LN, Thomas MR, Shanafelt TD. *Systematic review of depression, anxiety, and other indicators of psychological distress among US and Canadian medical students*. Acad Med 2006;81:354-73.
- [7] Firth J. *Levels and sources of stress in medical students*. Br Med J (Clin Res Ed) 1986;292:1177-80.
- [8] Stewart SM, Lam TH, Betson CL, et al. *A prospective analysis of stress and academic performance in the first two years of medical school*. Med Educ 1999;33:243-50.
- [9] Willcock SM, Daly MG, Tennant CC, et al. *Burnout and psychiatric morbidity in new medical graduates*. Med J Aust 2004;181:357-60.
- [10] Polychronopoulou A. *Perceived sources of stress among Greek dental students*. J Dent Educ 2005;69:687-92.
- [11] Frick LJ, Frick JL, Coffman RE, et al. *Student stress in a three-year doctor of pharmacy program using a mastery learning educational model*. Am J Pharm Educ 2011;75:64.
- [12] Sherina MS, Lekhraj R, Nadarajan K. *Prevalence of emotional disorder among medical students in a Malaysian University*. Asia Pac Fam Med 2003;2:213-7.
- [13] Zaid ZA, Chan SC, Ho JJ. *Emotional disorders among medical students in a Malaysian private medical school*. Singapore Med J 2007;48:895-9.
- [14] Mohsin S, Hasan S, Malik S, et al. *Perceived stress, sources and severity of stress among medical undergraduates in a Pakistani medical school*. BMC Medical Education 2010;10:2.
- [15] Redwood SK, Pollak MH. *Student-led stress management program for first-year medical students*. Teach Learn Med 2007;19:42-6.
- [16] Gentile JP, Roman B. *Medical student mental health services*. Psychiatry (Edgmont) 2009;6:38-45.
- [17] Shapiro SL, Shapiro DE, Schwartz GE. *Stress management in medical education: a review of the literature*. Acad Med 2000;75:748-59.

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Prevalence survey of healthcare-associated infections and antimicrobial use at the University Hospital "Paolo Giaccone", Palermo, Italy

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Key words

Healthcare-associated infections • Antimicrobials drug resistance • Point Prevalence Survey

Summary

Introduction. Healthcare-associated infections (HAIs) and antimicrobial resistance are well known major public health threats. The first goal of our study was to describe the prevalence of HAI, while the second goal was to describe the antibiotic consumption at our University Hospital, "P. Giaccone" in Palermo, Italy.

Methods. A standardized methodology for a combined Point Prevalence Survey (PPS) on healthcare-associated infections (HAIs) and antimicrobial use in European acute care hospital developed by the European Centre for Disease Prevention and Control (ECDC) was piloted across Europe. The teaching Hospital "P. Giaccone" in Palermo, Italy, participated in the study.

Results. Out of 328 surveyed patients, 12 (3.6%) had an HAI and 159 (48.5%) were receiving at least one antimicrobial agent. Prevalence results were highest in intensive care units, with 17.6% patients with HAI. Bloodstream infections represented the most common type (50%) of HAI. Surgical prophylaxis was the indication for antimicrobial prescribing in 59 (37.1%) out of 159 patients and exceeded 24 hours in 54 (91.5%) cases.

Discussion. The results suggest that in our hospital there was a frequent and inappropriate use of antimicrobials, especially in the setting of surgical prophylaxis.

Introduction

Healthcare-associated infections (HAIs) are an important cause of morbidity and mortality worldwide [1]. The annual prevalence of HAIs in Italy is estimated to range between 5% and 8% by year. The most frequent HAIs are the lower respiratory tract infections (LRTIs), followed by urinary tract infections (UTIs), bloodstream infections (BSIs) and surgical site infections (SSIs) [1]. In our country about 450,000-700,000 patients acquire at least one HAI during hospital stay [1-3]. Thus, HAI's surveillance is recognized as a crucial activity for the prevention and control programs.

Excessive and inappropriate use of antibacterial drugs is a major public health problem worldwide, since it is associated to an alarming increase in drug resistance and adverse drug reactions and causes also huge economical costs [4]. Available international and national data about amount and pattern of antibiotic use in both community and hospital setting are not always sufficiently informative because national databases may use different and no standardized methods to measure antibiotic use. To this aim, PPS (Point Prevalence Survey) can provide baseline information about occurrence and distribution of HAI and antibiotic use in healthcare institutions.

On September 2011, the teaching Hospital "Paolo Giaccone" of Palermo, Italy, participated to the European-scale Prevalence Survey for the Surveillance of Healthcare-Associated Infections (HAI) and Antimicrobial Use in Acute Care Hospitals [2]. This project was implemented by the European Center for Disease Prevention and Control (ECDC) through a protocol providing a standardized methodology to the Member States and the participating hospitals.

The specific objectives of Prevalence Survey were to estimate the total burden of HAI and antimicrobial use, by applying a standardized European methodology, and to disseminate the results at a local, regional, national and EU level.

Methods

SETTING

The Teaching Hospital "P. Giaccone" of Palermo, Italy, is a tertiary care hospital with 575 beds, of which 497 reserved to acute and 16 to intensive care, with nearly 20,400 admissions annually. The number of patient-days per year is about 151,700. This hospital in the European-scale Prevalence Survey of Healthcare-Associated Infections and Antimicrobial Use accounted for western Sicily.

POINT PREVALENCE SURVEY (PPS)

The standardized protocol for a combined Point Prevalence Survey (PPS) on healthcare-associated infections (HAIs) and antimicrobial use that we used, represents the final protocol (version 4) defined in 2011-2012 by ECDC, which carried out a review of 17 national or regional point prevalence surveys of HAI (and antimicrobial use) in European countries (ECDC Annual Epidemiological report 2008) [2, 5, 6].

A series of inclusion requirements had to be met. All acute care units were included, but long-term care units, accident and emergency (A&E) Departments, patients admitted to same-day treatment or surgery, the outpatient departments, emergency room and dialysis were ruled out. Data recovered for each patient aimed to identify an active HAI and/or the use of antimicrobial drugs at the time of the survey. Data had to be collected in a single day for each ward/unit. The total time frame of data collection for all units of a single hospital had not to exceed 2-3 weeks. Data were collected using two forms: "Hospital data form" including general information on the type of surveyed hospital and "Patient data form" subdivided in three parts: the first one dedicated to demographic and clinical data, the second one describing antimicrobials use and the last one regarding HAI.

In the pilot study, ECDC recommended that data collection was to be carried out by experienced staff in reading patient charts/notes and in HAIs' identifying (e.g. infection control professionals, clinical microbiologists, infectious disease specialists). In particular, in our survey, data collectors (HAIs' prevention and surveillance staff, residents) were previously trained by the national PPS coordinators to become familiar with protocol and case definition.

CASE DEFINITION

European case definition for HAI was used according to previous surveillance projects (HELICS or other European projects), whereas case definitions from the National Healthcare safety, Centers for Disease Control and Prevention (CDC), were otherwise used. In the HAIs section, data on microorganisms and their resistance phenotype were collected. Only results that were already available at the time of the survey were included. Data were also collected for patients showing an active HAI on the day of the survey. A HAI was defined as active when:

- signs and symptoms fulfilled the survey definitions of HAI and were present on the survey date
- or
- signs and symptoms fulfilling the survey definitions of HAI had been present in the past and the patient was still receiving treatment on the survey date.

DEFINITIONS OF ANTIMICROBIAL USE DATA

The antimicrobial-related informations were only collected if patient was receiving antimicrobials at the time of the survey or alternatively had an active HAI. Both generic and brand names were allowed. The drugs in-

cluded the ATC classes J01 (antibacterials), J02 (antifungals) and J04 (antimycobacterials). The route of administration was also recorded. The patients could receive systemic antimicrobials for:

- community-acquired infection (CI): all infections already present at admission except for those correlated to a previous hospitalization;
- healthcare infection acquired in long term care facility or chronic care hospital (LI);
- acute hospital acquired infection (HI): this also applied to hospital-acquired infections occurring after discharge and for occupational infections among staff of the structure;
- Surgical Prophylaxis (SP): any single dose of an antimicrobial agent given within the 24-hour period before 8:00 am on the day of the survey. This time window for surgical prophylaxis allowed for distinguish single dose prophylaxis, one day prophylaxis or prophylactic doses given over more than one day;
- Medical Prophylaxis (MP): it included antibiotic therapy administered to prevent a disease or its recurrence;
- other indications (e.g. use of erythromycin as a prokinetic agent);
- unknown indication/reason (assessed during PPS);
- unknown/missing information on indication not verified during PPS.

Results

A total of 328 patients, of whom 161 females (49.1%), were included in the PPS. Twenty-one patients were infants aged 0-10 months. The mean age of the remaining 307 patients was 61.8 [standard deviation (SD) 19.4] years for females and 60.4 (SD 18.8 years) for males. One hundred ninety patients out of 328 were hospitalized in clinical medicine departments (57.9%), 112 in surgical departments (34.1%) and 26% in intensive care units (ICUs).

Twelve (3.6%) out of the 328 patients had an HAI. One patient had a triple healthcare infection, so the total burden of HAIs was 14. BSIs represented the commonest type of HAI (7 out of 14), followed by UTIs (four out of 14), pneumonia, decubitus ulcer and SSIs. One (7.1%) central vascular catheter (CVC)-related infection was also identified. All UTIs were occurring in patients with urinary catheter. All of the six BSIs arose in patients with at least an invasive device in situ (CVC and/or peripheral vascular catheter, PVC) within for at least 48-hour. Prevalence was highest in ICUs (17.6%). In the department of gastroenterology two out of 17 patients had an active infection at the moment of survey similarly to the Department of Clinical Medicine Respiratory, where one out of nine patients had an HAI. The remaining infections were detected in the General Medicine ward (three out of 33 patients), Orthopedics and Traumatology ward (one out of 11 patients), Hematology (one out of 14 patients) and Emergency Surgery ward (one out of 20 patients had an HAI).

Overall, 159 (48.5%) out of 328 of the surveyed patients were prescribed one or more antimicrobials. Among the patients receiving at least one antimicrobial, 98 (61.6%) out of 159 were administered with a single agent, 47 (29.5%) two and 14 (8.8%) received three agents. Overall 59 (37.1%) patients were prescribed antibiotics for surgical prophylaxis, 58 (36.5%) for a community infec-

tions, 27 (17.0%) for medical prophylaxis, 12 (7.5%) for HAI, three (1.9%) for LI (Tab. I).

In 36 out of 73 (49.3%) of the cases of SP, cephalosporins were used, alone or in combination with other agents, followed by quinolones 16.4% (12 cases) and metronidazole 13.7% (10 cases) (Tab. II). Among 3rd

Tab. I. Pattern of administration of antibiotics and indications of treatment in the surveyed patients during the PPS, 2011.

Indication of treatment	Treated patients (n. 159)		Number of antimicrobials used			
			1		> 1	
	n*	%□	n≠	% ¹	n≠	% ¹
Medical prophylaxis	27	16.9%	18	66.6%	9	33.4%
Surgical prophylaxis	59	37.1%	42	71.2%	17	28.8%
Community infection	58	36.5%	36	62.1%	22	37.9%
Healthcare-associated infection	12	7.5%	2	16.6%	10	83.4%
Long-term hospital-acquired infection	3	2.0%	–	–	3	100.0%

ECDC: European Centre for Disease Prevention and Control.

* Total of patients treated for relative indication.

Percent of the total.

≠ Patients receiving the indicated number of antimicrobial agents for each indication of treatment.

¹ Percent each within category.

Tab. II. Distribution of antimicrobial agents (ATC* 4th levels) by main indication for use, ECDC pilot point prevalence survey, 2011 (n = 223 antimicrobial agents).

	All indication	Treatment	Surgical prophylaxis	Medical prophylaxis
	n (%)	n (%)	n (%)	n (%)
Antimicrobial agents, total	223 (100)	113 (100)	73 (100)	37 (100)
Top antimicrobial agents at ATC 4th level				
Fluoroquinolones (J01MA)	56 (25.1)	34 (30.1)	12 (16.4)	10 (27.0)
Third-generation cephalosporins (J01DD)	55 (24.6)	12 (10.6)	35 (47.9)	8 (21.6)
First-generation cephalosporins (J01DB)	1 (0.4)	–	1 (1.4)	–
Nitroimidazole derivates (P01AB)	27 (12.1)	11 (9.7)	10 (13.7)	6 (16.2)
Combinations of penicillins, incl.β-lactamase inhibitors (J01CR)	18 (8.1)	11 (9.7)	4 (5.4)	3 (8.1)
Combination of sulfonamides and trimethoprim (J01CA)	10 (4.4)	4 (3.5)	2 (2.7)	4 (10.8)
Macrolides (J01FA)	9 (4.0)	6 (5.3)	–	3 (8.1)
Glycopeptide antibacterials (J01XA)	9 (4.0)	9 (7.9)	–	–
Aminoglycosides (J01GB)	8 (3.6)	4 (3.5)	4 (5.4)	–
Penicillins, extended spectrum without anti-pseudomonal activity (J01CA)	8 (3.6)	6 (5.3)	2 (2.7)	–
Carbapenems (J01DH)	7 (3.1)	6 (5.3)	1 (1.4)	–
Antidiarrheals, intestinal anti-inflammatory/anti-infective agents (A07AA)	5 (2.2)	3 (2.6)	–	2 (5.4)
Triazole derivates (J02AC)	3 (1.3)	3 (2.6)	–	–
Antimycobacterials for treatment of tuberculosis (J04A)	2 (0.8)	2 (1.7)	–	–
Tetracyclines (J01AA)	1 (0.4)	1 (0.8)	–	–
Other antibacterials (J01XX)	3 (1.3)	1 (0.8)	1 (1.4)	1 (2.7)

* Anatomical Therapeutic Chemical

generation cephalosporins, ceftriaxone, was the most frequently used.

In MP the quinolones were administered at a rate of 27.0% (10 out of 37), cephalosporins of 21.6% (8 out of 37) and metronidazole of 16.2% (6 out of 37). Of the 59 patients treated for surgical prophylaxis, only in one case the antibiotics were administered in single dose (1.7%), while in four cases for one day (6.8%) and in all the other cases (54 cases, 91.5%) for more than one day. In these last cases, the antibiotic administration was also prolonged after surgery. One antibiotic was administered to 37 (68.5%) out of 54 patients, while more than one antibiotic was given to the remaining 17 (31.5%).

Data on causal agents and their respective resistant phenotype were included only if they were already available on the date of the survey. Therefore, data on the species of microorganisms causing HAI were unavailable, except for six cases. *Enterobacteriaceae* (*E. cloacae*) and enterococci (*E. faecalis* and *E. faecium*) were associated with urinary tract, blood, decubitus ulcers, including both superficial and deep infections and SSI. One BSI was associated with *Cytomegalovirus*. *Candida albicans* and *Acinetobacter baumannii* were also identified. This last one affected three infection sites in a single patient (decubitus ulcer, bloodstream and urinary tract). Resistance to third generation cephalosporin and carbapenem was reported for *E. cloacae*.

Discussion

HAIs are a serious problem, contributing heavily to the burden of the hospital costs [7]. These infections have a negative impact on patient because of the worsening of underlying medical condition and the increased morbidity and mortality [7].

The rapidly escalating prevalence of antimicrobial resistance is a global concern. The reduced susceptibility to most current of available antimicrobial agents coupled with the progressive shortage of newly approved compounds is a worrisome situation [7]. Major problems are encountered for a growing number of Gram-positive organisms (i.e., *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus* spp.) and Gram-negative pathogens (i.e., *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*) [7]. Serious infections caused by resistant bacteria do not respond to therapy and are often associated with worse outcomes, including increased rates of complications, additional costs, higher associated mortality rates and prolonged hospital stays [8].

HAIs' prevalence of 3.6% observed in our survey is lower than that reported in other European studies and that observed in 2008 (6.7%) in the same hospital. The limits of the HAI's estimate by PPS are well known. However, because case-mix and admission rules are not changed in the last years, it can be presumed that some prevention and control interventions have been to some extent effective. In particular

some guidelines were issued to implement infection control procedures, such as Antibiotic perisurgical pro-

phylaxis in adults, Ambulances hygiene, handwashing (Clean care is safer care), Isolation measures in the AOUP P. Giaccone, Palermo, isolation of patients with colonization/infection by multi-resistant pathogens. So healthcare workers training programs were carried out as well.

The European prevalence has been estimated to be 7.1% by ECDC, based on a review of 30 national or multi-centre PPS in 19 countries in its annual epidemiological report for 2008 [4, 6]. However an important issue that should be considered for the interpretation of the epidemiological results of this and future surveys is the standardization of data collection in participating hospitals to compare results between hospitals, regions and countries. Indeed, HAI prevalence may depend on differences in methodology and patients case-mix and not only on performance variations.

High rates of antibiotic usage were observed in our hospital. In particular our survey revealed use of broad spectrum antibiotics, sometimes combined in multidrug protocols, because of emergence of antibiotic-resistant microorganisms [9]. On the other hand, due to the widespread diffusion of MDR organisms in our healthcare settings, it is often necessary to start an initial antibiotic therapy with multidrug protocols or last resource molecules, such as colistin. New diagnostic methods that allow to obtain microbiological confirmation in short time could be very useful. It is known that the excessive and unnecessary use of antibiotics is the main driving force of the high rates of drug resistant infections we are seeing in recent years [10, 11].

The results of this survey confirm those of a parallel analysis with another classical approach in which the antibiotic use in the hospital was measured by evaluating the numbers of defined daily doses (DDD) (Malta R et al., 2010) for 100 bed days or for number of admissions. The DDD consumed in 2011 were in fact 82.06 per 100 bed-days and 5.41 per admission. For the surgical wards, overall the DDD were 94.00 per 100 bed-days and 5.98 per admission; quinolones accounted for 25.6% of the DDD consumed, cephalosporins for 23.7%, penicillins for 16.2% and metronidazole for 14.4%. Moreover, DDD consumed for 100 bed-days in our hospital proved to be higher than those observed in a sample of five hospitals in Emilia Romagna despite increasing by 18% in the three years period, 64.9 DDD/100 bed days in 2002 to 76.7 in 2004 [9, 12, 14].

Results showed also that a significant area of antibacterial overuse was SP. According to the international consensus, antibiotic prophylaxis in surgery involves the administration of an antibacterial agent for a very short time, temporally located just before the beginning of intervention [12]. However, our study revealed that the antibiotics were used for longer intervals of times. A reason of inappropriate antibacterial drug use was the frequent administration as SP of drugs, such as 3rd generation cephalosporins, which are not recommended for surgical prophylaxis and should be reserved to the treatment of severe active infections.

Our study has some limitation. Indeed the PP study design has some inherent limits, including reduced periods

of observation and the possibility of obtaining biased results. It has the advantage to be very economical in terms of both time and human and financial resources. Repeated PPS represent a more feasible alternative for hospital-wide surveillance of all HAIs, while still allowing the estimation of HAIs burden in acute hospitals, and helping to prioritize areas requiring interventions [1]. Therefore, although it was carried out in full compliance with the European protocol, the study has likely some limitations inherent to the study design. Continuous surveillance, especially prospective active surveillance, is the gold standard to improve patient safety. The HAI prevalence rate detected in our PPS was lower than expected. However, our investigation highlighted a

large misuse of antibacterial drugs, in particular in the area of SP. Prophylactic use of antibiotics in surgery is an example of how a potentially useful practice can be misused. More in general, inappropriate antibiotic use can lead to increased morbidity, prolonged hospital stay and need for more expensive drugs [13, 14]. The main aim of antibiotic stewardship is to bring a change in prescribing which could lead to control of drug resistance, decreased costs and improved quality of antibiotic use. Approaches that have to be, also locally, adopted include educational programs, development of prescribing guidelines (e.g. *Clean Care is Safer Care*, WHO) [15], monitoring of drug resistance patterns, turning to restrictive hospital formularies [16, 17].

References

- [1] Lanini S, Jarris WR, Nicastrì E, et al. *Healthcare-associated infection in Italy: annual point-prevalence surveys, 2002-2004*. Infect Control Hosp Epidemiol 2009;30:659-65.
- [2] CDC/NHSN Surveillance. *Definition of healthcare-associated infection and criteria for specific types of infections in the acute care setting*. AM J Infect Control 2008;36:309-32.
- [3] Zotti CM, Messori Ioli G, Charrier L, et al. *Hospital-acquired infections in Italy: a region wide prevalence study*. J Hosp Infect 2004;56:142-9.
- [4] Brusaferrò S, Regattin, Faruzzo A, et al. *Surveillance of hospital-acquired infections: a model for settings with resource constraints*. Am J Infect Control 2006;34:362-6.
- [5] Kuijper EJ, Coignard B, Tüll P; the ESCMID Study Group for Clostridium difficile (ESGCD); EU Member States and the European Centre for Disease Prevention and Control (ECDC). *Emergence of Clostridium difficile-associated disease in North America and Europe*. Clin Microbiol Infect 2006;12(Suppl. 6):2-18.
- [6] Geffers C, Baerwolff S, Schwab F, et al. *Incidence of healthcare-associated infections in high-risk neonates: results from the German surveillance system for very-low-birthweight infants*. J Hosp Infect 2008;68:214-21.
- [7] Furtado GH, Nicolau DP. *Overview perspective of bacterial resistance*. Expert Opin Ther Pat 2010;20:1273-6.
- [8] Collignon P. *Clinical impact of antimicrobial resistance in humans*. Rev Sci Tech 2012;31:211-20.
- [9] Mazzeo F, Capuano A, Motola G, et al. *Antibiotic use in an Italian University Hospital*. J Chemother 2002;14:332-5.
- [10] Zarb P, Coignard B, Griskeviciene J, et al. *The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use*. Euro Surveill 2012;17(46). pii: 20316.
- [11] Malta R, Di Rosa S, D'Alessandro N. *Aspetti etici e controllo di gestione dei farmaci antibiotici antibatterici*. Italian Journal of Medicine 2010;4:137-44.
- [12] Vaccheri A, Silvani MC, Bersaglia L, et al. *A 3 year survey on the use of antibacterial agents in five Italian hospitals*. J Antimicrob Chemother 2008;61:953-8.
- [13] Schön T, Sandelin LL, Bonnedahl J, et al. *A comparative study of three methods to evaluate an intervention to improve empirical antibiotic therapy for acute bacterial infections in hospitalized patients*. Scand J Infect Dis 2011;43:251-7.
- [14] Müller-Peabody B, Muscat M, Pelle B, et al. *Increase and change in pattern of hospital antimicrobial use*. Denmark 1997-2001. J Antimicrob Chemother 2004;54:1122-6.
- [15] Pittet D, Donaldson L. *Clean care is safer care: a worldwide priority*. Lancet 2005;366:1246-7.
- [16] Bassetti M, Topal J, Di Biagio A, et al. *The organization of infection control in Italy*. J Infect 2001;48:83-5.
- [17] Porretta A, Giuliani L, Vegni FE, et al. *Prevalence and patterns of antibiotic prescribing in Italian hospitals*. Infection 2003;31(Suppl. 2):16-21.

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ORIGINAL ARTICLE

Staphylococcus aureus infections in children in an Iranian referral pediatric Hospital

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Key words

Staphylococcus aureus • Methicillin-Resistant • Children

Summary

Introduction. *Staphylococcus aureus* is associated with various infections ranging from skin and soft tissues such as surgical site infections and abscesses to lower respiratory tracts and bloodstream. The aim of this study was to evaluate underlying condition of patients with *S. aureus* infections in an Iranian referral pediatric Hospital.

Material and methods. Information was extracted retrospectively from the medical records of patients who were diagnosed with *S. aureus* infections. Data obtained about the study subjects included basic demographics, reason for admission, culture site, length of hospital stay, and methicillin susceptibility.

Results. The underlying condition of patients with *S. aureus* infection during November 2011 and March 2013 were included in the study. The most frequent diagnosis in patients with *S.*

aureus infection was jaundice (12%), abscess (10%), cellulitis (10%), wound infection (8%), septic arthritis (7%) and seizure (5%). Wound was the most common infection sites among all subjects 34/98 (35%) followed by blood (20/98, 20%) as well as skin and soft tissue (19/98, 19%). The proportion of MRSA infections among all *S. aureus* isolates was 79% (77/98) during the study period. In addition, 58/74 (78%) met the definition of Hospital-Associated Methicillin-Resistant *S. aureus* (HA-MRSA) infections and the rest; 20/24 patients (83%), were classified as Community-Associated Methicillin-Resistant *S. aureus* (CA-MRSA).

Conclusion. In our study, the high frequency of MRSA was found not only in HA *S. aureus* but also in CA *S. aureus* isolates; therefore, the strategic goals to optimize antimicrobial use including

Introduction

Staphylococcus aureus is associated with various infections ranging from skin and soft tissues to lower respiratory tracts and bloodstream and even life-threatening infections such as endocarditis and osteomyelitis [1, 2]. There are both a rapid emergence of nosocomial *S. aureus* infection and increasing prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in the hospital and community [3].

Hospitalized patients show a high frequency of *S. aureus* infections due to their weak immune system as well as frequent injections and catheterizations mainly in intensive care units (ICU) [1, 4].

The aim of this study was to evaluate underlying condition of patients with *S. aureus* infections in an Iranian referral pediatric Hospital.

Material and methods

This study was conducted at a Children medical center, an Iranian referral Hospital during November 2011 and March 2013.

Information was extracted retrospectively from the medical records of patients who were diagnosed with *S. aureus* infections. Data which was obtained about the study subjects included basic demographics, reason for admission, culture site, length of hospital stay, and methicillin susceptibility.

Hospital-Associated Methicillin-Resistant *Staphylococcus aureus* (HA-MRSA) infection was defined as occurring in a patient whose *S. aureus* isolate was cultured more than 48 h after admission, who had a history of hospitalization or residence in a long-term healthcare facility within 6 months prior to the culture date. Patients with none of the above conditions that their culture samples were obtained prior to admission or during the first 48 h of hospitalization from patient lacks the following health care-associated MRSA risk factors: hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient were classified as having Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) infection [5].

Clinical isolates were identified as *S. aureus* by Gram stain, colonial morphology, catalase and coagulase test. Susceptibility to oxacillin was performed using oxacillin disk according to the performance standards of the Clinical and Laboratory Standards Institute (CLSI) [6].

Statistical analyses

The Statistical Package for the Social Sciences (Windows version 16.0; SPSS Inc, Chicago, US) was used for all analyses. Descriptive statistics were used to summarize patient variables.

Results

In this study, records of 98 *S. aureus* infected patients who were diagnosed during November 2011 and March 2013 were analysed. The mean age of the patients was 3.3 ± 2.45 years (range from 1 day to 13.8 year) and the male to female ratio was 1.2.

S. aureus isolates were frequently isolated from patients who were suffered from jaundice (12%), abscess (10%), cellulitis (10%), wound infection (8%), septic arthritis (7%) and sezeire (5%).

The most common infection sites which *S. aureus* strains were isolated were wound (34/98,35%) following by blood (20/98, 20%) and skin and soft tissue (19/98, 19%).

The proportion of MRSA infections among all *S. aureus* isolates was 79% (77/98) during the study period. In addition, 58/74 (78%) met the definition of HA-MRSA infections and the rest; 20/24 patients (83%), were classified as CA- MRSA.

Among all *S. aureus* isolates, 24 CA- *S. aureus* isolates were associated generally with the wound, skin and soft tissue infections infection. The sites of CA-MRSA and HA-MRSA infections is shown in Table I.

Discussion

The rate of MRSA has been increased considerably over the last decade [7], and are mainly associated with skin and soft tissue infections particularly abscesses and cellulitis [8].

According to our results, the high frequency of MRSA was found not only in HA- *S. aureus* isolates but also in CA- *S. aureus* isolates. CA-MRSA strains appear to have rapidly disseminated among the general population and affect patients with and without exposure to the health care environment.

The frequency of MRSA infections among all *S. aureus* isolates in this study was more than other studies [5, 9-12]. Huang et al. reported that 42% of the *S. aureus* isolates were MRSA (55.1% HA-MRSA and 44.9% CA-MRSA) [5]. Aminet al. reported that CA-MRSA infections represented 31.5% of CA- *S. aureus* infections, while HA-MRSA accounted for 52.6% of HA- *S. aureus* infections [9].

The rapid emergence of MRSA in the recent years might be due to the increasing consumption of antibiotics, long time of hospitalization, failures of hospital hygiene and even selective and mobile genetic elements [3, 13-16]. Soriano et al. has been reported a 1.8 fold increase in MRSA isolation in patients who were prescribed more than 2 antibiotics in the last 180 days [17].

In our study, *S. aureus* isolates were commonly associated with wound, bloodstream and skin, and soft tissue infections. Caini et al. reported that more than 50% of all reported HA-MRSA isolates belonged to surgical site as well as skin and soft tissues in Hungarian patients during 2005-2010 [18].

In our study, the frequency of MRSA causing bloodstream infections was 60% (12/20). In the previous studies which were conducted in our hospital, the rates of MRSA was 60% and 79% during 1996–2000 [19] and 2001-2005 [20], respectively.

In this study, the rate of wounds MRSA isolates was higher than other reports (26/34, 76%) [2, 21]. In Wolk et al. [2] and Borgundvaag et al. studies [21], the prevalence of wound MRSA isolates was 30% and 32%, respectively. Skin and soft tissue consider as one other common infection sites [19] that was found in 25% of patients. This rate was less than Huang et al. reports [5]. Drug resistance has continued to emerge particularly in intensive care units (ICUs) [14, 15]. In this study, a high frequency of MRSA was found in Pediatric Intensive Care Unit (PICU) and Neonatal Intensive Care Unit (NICU) (80% and 77%, respectively). Minimizing the antibiotic pressure and duration of empiric therapy as well as hand hygiene is essential for controlling the emergence of these resistant strains in the hospitals [22, 23].

Tab. I. Sites of CA-MRSA and HA-MRSA infections.

		Wound	Bloodstream	Skin and soft tissue	Eye	Respiratory tract	Other*	Total
CA-MRSA	n	6	2	6	4	0	2	20
	%	33	13	25	21	0	8	100
HA-MRSA	n	20	12	9	7	8	2	58
	%	35	23	18	11	11	3	100

* Includes urine tract and bone marrow aspiration

CA: Community-Associated

HA: Hospital-Associated

Moreover, identifying of the important factors contributing to acquisition and transmission of *S. aureus* infection as well as antibiotic stewardship programs should imply in our hospital.

The spread of MRSA is usually occurring by transient carriage on the hands of health care workers. In our hospital due to high rate of MRSA isolates, improvement in the adherence of health care workers to hand-hygiene guidelines should perform. In addition, aseptic technique such as waterless antiseptic hand rubs, use of sterile gowns and gloves must be practiced. In addition,

series of appropriate guidelines for antimicrobial use and a chart audit should be developed.

In conclusion, in our study, the high frequency of MRSA was found not only in HA *S. aureus* but also in CA *S. aureus* isolates; therefore, the strategic goals to optimize antimicrobial use including optimizing choice and duration of empiric therapy, monitoring and providing feedback regarding antibiotic resistance, prevention of antimicrobial resistant transmission as well as basic infection control policies and procedures are recommended.

References

- [1] Valaperta R, Tejada MR, Frigerio M, et al. *Staphylococcus aureus* nosocomial infections: the role of a rapid and low-cost characterization for the establishment of a surveillance system. *New Microbiol* 2010;33:223.
- [2] Wolk D, Struelens M, Pancholi P, et al. *Rapid detection of Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) in wound specimens and blood cultures: multicenter preclinical evaluation of the Cepheid Xpert MRSA/SA skin and soft tissue and blood culture assays.* *J Clin Microbiol* 2009;47:823-6.
- [3] Hsueh P-R, Teng L-J, Chen W-H, et al. *Increasing prevalence of methicillin-resistant Staphylococcus aureus causing nosocomial infections at a university hospital in Taiwan from 1986 to 2001.* *Antimicrob Agents Chemother* 2004;48:1361-4.
- [4] Campos GB, Souza SG, LOB O, et al. *Isolation, molecular characteristics and disinfection of methicillin-resistant Staphylococcus aureus from ICU units in Brazil.* *New Microbiol* 2012;35:183.
- [5] Huang H, Flynn NM, King JH, et al. *Comparisons of community-associated methicillin-resistant Staphylococcus aureus (MRSA) and hospital-associated MRSA infections in Sacramento, California.* *J Clin Microbiol* 2006;44:2423-7.
- [6] Wikler MA. *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement.* Clinical and Laboratory Standards Institute; 2010.
- [7] Kopp BJ, Nix DE, Armstrong EP. *Clinical and economic analysis of methicillin-susceptible and-resistant Staphylococcus aureus infections.* *Ann Pharmacother* 2004;38:1377-82.
- [8] Eady EA, Cove JH. *Staphylococcal resistance revisited: community-acquired methicillin resistant Staphylococcus aureus-an emerging problem for the management of skin and soft tissue infections.* *Curr Opin Infect Dis* 2003;16:103-24.
- [9] Amin N, Faidah HS. *Methicillin-resistant Staphylococcus aureus in the western region of Saudi Arabia: prevalence and antibiotic susceptibility pattern.* *Ann Saudi Med* 2012;32:513-6.
- [10] Mamishi S, Mahmoudi S, Sadeghi R, et al. *Genotyping of Staphylococcus aureus strains among healthcare workers and patients in the tertiary referral Children's Medical Hospital in Tehran, Iran.* *Br J Biomed Sci* 2011;69:173-7.
- [11] Lamy B, Laurent F, Gallon O, et al. *Antibacterial resistance, genes encoding toxins and genetic background among Staphylococcus aureus isolated from community-acquired skin and soft tissue infections in France: a national prospective survey.* *Eur J Clin Microbiol Infect Dis* 2012;31:1279-84.
- [12] Köck R, Mellmann A, Schaumburg F, et al. *The epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) in Germany.* *Dtsch Arztebl Int* 2011;108:761.
- [13] Evans M. *Selection of oxacillin resistance in oxacillin-sensitive Staphylococcus aureus through exposure to ciprofloxacin but not piperacillin/tazobactam.* *Eur J Clin Microbiol Infect Dis* 2002;21:621-3.
- [14] Weinstein RA. *Controlling antimicrobial resistance in hospitals: infection control and use of antibiotics.* *Emerg Infect Dis* 2001;7:188.
- [15] Goldmann DA, Weinstein RA, Wenzel RP, et al. *Strategies to Prevent and Control the Emergence and Spread of Antimicrobial-Resistant Microorganisms in Hospitals: A Challenge to Hospital Leadership.* *JAMA* 1996;275:234-40.
- [16] Shorr AF, Zilberberg MD, Micek ST, et al. *Prediction of infection due to antibiotic-resistant bacteria by select risk factors for health care-associated pneumonia.* *Arch Intern Med* 2008;168:2205.
- [17] Soriano A, Marco F, Martínez JA, et al. *Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant Staphylococcus aureus bacteremia.* *Clin Infect Dis* 2008;46:193-200.
- [18] Caini S, Hajdu A, Kurcz A, et al. *Hospital-acquired infections due to multidrug-resistant organisms in Hungary, 2005-2010.* *Euro surveillance: bulletin europeen sur les maladies transmissibles= European communicable disease bulletin* 2012;18(2).
- [19] Mamishi S, Pourakbari B, Ashtiani MH, et al. *Frequency of isolation and antimicrobial susceptibility of bacteria isolated from bloodstream infections at Children's Medical Center, Tehran, Iran, 1996-2000.* *Int J Antimicrob Agents* 2005;26:377-9.
- [20] Pourakbari B, Sadr A, Ashtiani MTH, et al. *Five-year evaluation of the antimicrobial susceptibility patterns of bacteria causing bloodstream infections in Iran.* *J Infect Dev Ctries* 2011;6:120-5.
- [21] Borgundvaag B, Ng W, Rowe B, et al. *Prevalence of methicillin-resistant Staphylococcus aureus in skin and soft tissue infections in patients presenting to Canadian emergency departments.* *CJEM* 2013;15:141-60.
- [22] Cheng VC, Tai JW, Wong ZS, et al. *Transmission of methicillin-resistant staphylococcus aureus in the long term care facilities in Hong Kong.* *BMC Infectious Diseases* 2013;13:205.
- [23] Pourakbari B, Rezaizadeh G, Mahmoudi S, et al. *Epidemiology of nosocomial infections in pediatric patients in an Iranian referral hospital.* *J Prev Med Hyg* 2012;53:204.

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Social and interpersonal relationship modifications after renal transplant. A statistic and epidemiologic evaluation

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Key words

Health-related quality of life • Kidney transplant • Epidemiological study

Summary

Introduction *Kidney and liver transplants are the most frequent transplantation procedures carried out in Italy. We report the result of an epidemiological study on kidney transplanted patients resident in the Province of Messina (Italy).*

Methods *Seventy-five patients were enrolled between June 2010 march 2011, interviewed and evaluated using an adapted Italian version short-form 36. Socio-economic characteristics, quality of life modifications and involvement in transplant-related charities were studied. The follow-up period was ranging between 52 and*

356 months. All subjects gave written informed consent and all results were analysed by chi-square test.

Results *No statistically significant differences were found between sexes, social and interpersonal relationship modifications.*

Discussion *The benefits obtained on quality of life after transplantation is the prerogative of a small percentage of patients and is related to medium and high socio-economic conditions. The possibility of avoiding the haemodialysis represents the primary benefit for the totality of patients.*

Introduction

The incidence and prevalence of chronic renal insufficiency and end-stage renal disease have constantly increased during the last ten years. The number of patients who need kidney transplant and other treatment has also increased. For haemodialysis patients, kidney transplant represent the gold standard of treatment to improve quality of life and to extend survival. A problem for renal transplant is organs shortage and consequently a long waiting list [1, 2].

Every year approximately 3000 organ transplants are carried out in Italy. In 2010, one thousand five hundred and twelve kidney (1512) and one thousand and two liver (1002) transplants (of which, 57 and 58 in Sicily) were performed. These are the most frequent transplants carried out on the Italian national territory. Other transplants in the same year were heart (273), lung (107) and pancreas (74). Generic epidemiological data were derived from the national ministerial internet site [3].

To the present date, there are approximately 10000 people waiting for a transplant: 7048 adults and 103 children for a kidney transplant, 1485 adults and 30 children for a liver transplant and 869 adults and 29 children for a heart transplant [4].

Organ failure represents a serious clinical problem with relevant social and interpersonal implications and occupational disadvantages for patients on a waiting list for a transplant. These patients are often incapacitated by asthenia and malaise because they are forced to live

for long periods waiting for an organ, with many discomfort disease-related and also life-style limitations (haemodialysis, diet), emotional disturbances such as depression, anxiety, rage and, in some cases, economic difficulties [5].

In clinical practice, kidney transplant from a living donor is considered better in increasing the patient's survival than that from a dead donor, however, only a few studies have previously compared the quality of life in these cohorts of patients [6, 7].

A recent Australian study assessed the comparative survival and economic benefits after kidney transplant from dead donor compared to dialysis [8]. Renal transplant, though still controversial in some health programs, has now gained a widespread acceptance about emotional opinions; special interest was devoted to the evaluation of the ethical issues of psychological distress and the improvements of the quality of life [9]. Psychological aspects have become increasingly important, as to a better quality of life is associated with less morbidity and mortality. Quality of life is defined as the value assigned to the lifespan modified by disability, functional conditions, perceptions and social-work opportunities in subjects with illnesses and injuries [10, 11].

We report the result of an epidemiological study on kidney transplanted patients resident in the Province of Messina (Sicily, Italy) in order to estimate the socio-economic characteristics, psychological distress and the modifications brought from the transplant to the health-related quality of life.

Material and methods

In April 2010 the list of kidney transplanted patients, with personal information and telephone numbers, was confidentially transferred to our Department according to the protection of personal data legislation. Between June 2010 and March 2011, a telephone interview was carried out. All transplant patients were included in this study. We recruited 81 kidney transplant patients, that underwent a transplant procedure in Italian and European hospitals; 6 (7.4%) of these patients were lost to follow-up and excluded. Transplants were performed from January 1981 to December 2006. All patients enrolled in this observational study gave written informed consent, and local ethics committee approved the study. Seventy subjects received an organ from a dead donor and five from a living donor. These 75 kidney transplant patients were all regularly followed up by the Nephrology Unit of the University Hospital of Messina. The enquiry form, developed from the Italian version of the short form 36, was adapted and administered, during the follow-up, to all patients. Follow-up period was variable between 52 and 356 months. In this form, the first section enquired about demographic data, disease history and surgical transplant centre details; the second asked about family status, socio-economic conditions, health-related well-being modifications; the final section evaluated chronic pharmacological treatment and recent laboratory tests. In all cases the body mass index was evaluated. The mean value was 28.1 with a standard deviation (SD) of 4.77 and a range between 20 and 38. All results were analysed by chi-square test with a confidence interval (CI) of 95%. The most relevant statistical events were also investigated by Kolmogorov-Smirnov test.

Results

The group of kidney transplanted patients comprised 75 patients, 46 (61.3%) males (M) and 29 (38.7%) females (F). The mean age was 52.6 years with a SD of 11.71 and a range between 21 and 77 years. In 42 (56%) M and 28 (37.3%) F, the transplanted organs came from a cadaver; only 5 patients (6.7%, 4M and 1F) benefited from a living donor transplant. Sixteen (21.3%) patients had a graft versus host disease that required a second transplant; in particular 14 had a single (9M/5F), and 2 a double transplant rejection. In a case of single immunoreaction allograft-related, a patient received the second organ from a living donor. For males the mean conventional haemodialysis treatment duration was 48 months (range 1-156) before the first transplant, 35 months (1-84 months) before the second transplant and 48 months (12-60) before the third transplant; for females this period was 56 months for the first transplant (7-144) and 53 months (24-120) for the second transplant. Immunosuppressive and corticosteroid treatment was patient-specific, according to the drug-tolerance. It was an adapted treatment in 41 (54.7%) men and 26 (34.7%) women, exclusively immunosuppressive in 4 (5.3%) men and

3 (4%) women and exclusively corticoid in only 1 (1.3%) man. Mean creatinemia level (normal value 0.5-1.4 mg/dl) was 1.64 (SD = 0.98, CI 95%, range 0.5-4.9) and mean azotemia level (10-50 mg/dl) was 68.75 (SD = 37.78, CI 95%, range 23-209). Kidney failure causes were: glomerulopathy related to systemic diseases (41.3%), chronic glomerulopathy (18.7%), renal malformations and polycystic disease (16%), hypertensive nephrosclerosis (14.7%), tubulointerstitial nephritis (5.3%), and obstructive uropathies and pyelonephritis (4%). Co-morbidity (arterial hypertension, diabetes mellitus insulino-independent, thyropathies, bradi- and tachyarrhythmias, cholecystopathies and dyslipidemias) was present in 62 patients (82.7%).

One male-patient with an elevated level of creatinemia returned in conventional haemodialysis and is waiting for a new transplant. Socio-economic characteristics, health-related quality of life modifications and involvement in transplant-related charities are detailed respectively in Table I. No statistically significant differences were found between sexes, social and interpersonal relationship modifications. Subjects with medium and high socio-economic conditions showed higher appreciation in the quality life enquiry form compared to those with low income; however this didn't reach statistical significant ($p > 0.5$). The results of statistical analysis between pre-transplant depression episodes and social relationship modifications (chi-square = 1.77, $P = 0.778$, CI 95%) were not casual for absent and sporadic (1-3 episodes per year) events, and casual for frequent (4-6 per year); as well as in association with interpersonal relationship modifications (chi-square = 2.66, $P = 0.616$, CI 95%). The correlations between absent and sporadic pre-transplant depression episodes and social relationship modifications (respectively $D = 0.356 > 0.238$ and $D = 0.484 > 0.361$, CI 95%) as well as in association with interpersonal relationship (respectively $D = 0.267 > 0.235$ and $D = 0.484 > 0.361$, CI 95%) better investigated by Kolmogorov-Smirnov test.

Discussion and conclusion

Dialysis and renal transplant increase survival in patients with chronic renal failure; however it is not possible to compare between these treatments. The main aim of a renal transplant is to improve health-related quality of life and increase survival rates [12, 13]. Renal transplant requires an adequately stable clinical status, necessary to minimise post-operative complications, to reduce adverse effects secondary to immunosuppressant and to deal with a potential unsuccessful transplant.

Recent literature [14] suggests that renal transplant from a dead donor could improve survival rates in specific groups of patients with chronic renal failure, in patients believed to be adequately clinically and physically stable. On the other hand some studies [15] suggest that patients who receive an organ from a living donor have longer survival compared to patients undergoing dialysis

Tab. I. Socio-economic characteristics of the interviewed patients and health-related quality of life modifications.

	%	Total	Males	Females
Housing				
urban	76%	57	37	20
suburban	22.7%	17	9	8
rural	1.3%	1	0	1
Head of household				
	61.3%	46	39	7
Marital status				
married	72%	54	40	14
single	20%	15	4	11
widowed	6.7%	5	1	4
divorced	1.3%	1	1	0
Household income				
Low (< 14000 €)	49.3%	37	20	17
middle (> 14000 <30000 €)	41.3%	31	21	10
middle-high (> 30000 €)	9.4%	7	5	2
Depression episodes (before transplant)				
absent	60%	45	28	17
sporadic (1-3 episodes/year)	25%	19	12	7
frequent (4-6 episodes/year)	14%	11	6	5
Smoking				
	62.7%	47	28	19
Alcohol/Drug abuse				
	0%	0	0	0
Convictions				
	0%	0	0	0
Social relationships modifications				
considerable	60%	45	28	17
good	4%	3	2	1
sufficient	8%	6	5	1
insufficient	18.7%	14	9	5
none	9.3%	7	2	5
Interpersonal relationships modifications				
considerable	53.4%	40	28	12
good	1.3%	1	0	1
sufficient	16%	12	6	6
insufficient	12%	9	5	4
none	17.3%	13	7	6
Involvement in transplant-related charities				
yes	49.3%	37	24	13
no	50.7%	38	22	16

or those who receive an organ from a dead donor. New advances in immunosuppressive treatment, diagnosis and treatment of infectious complications; studies on ischaemic damage show a progressive improvement of short and long-term outcomes [16]. There is little agreement in the literature about quality of life improving following a transplant, however a recent cohort study [12] found a significant improvement in the quality of life in non-diabetic patients following a renal transplant from a living donor, compared to patients who were treated with dialysis. Another recent paper focused on psychological correlates (such as mood, anxiety and emotional symptoms) and their relationship with improved renal function following a transplant [17, 18], highlighting interesting findings needing replication.

Our findings suggest that quality of life after kidney transplant is more improved only for a small percentage of patients, generally with a declared higher income. Life before a transplant, considered by patients as a “forced routine”, with the associated restlessness and anxiety (waiting for the availability of an organ) becomes, in some cases, a post-transplant life with tight dependence on pharmacological treatment. However, in the majority of cases, patients admitted benefits of the transplant and real improvements in quality of life (thanks to not having to rely on haemodialysis). Patients in this study who received an organ from a live donor, had higher anxiety compared to patients who received an organ from a dead donor; however we could not draw inferences due to the small number of patients in the former group.

In the last 15 years, the number of transplant procedures for kidney failure have increased despite little progress in organ donation policy development and limited organ

availability. Quality of life is a very important determinant of long term outcomes following a transplant for patients with a long life expectancy.

References

- [1] Cameron JS. *Renal transplantation in the elderly*. Int Urol Nephro 2000;32:193-201.
- [2] Cortesini R, Renna-Molajoni E, Cinti P, et al. *Tailoring of immunosuppression in renal and liver allograft recipients displaying donor specific T-suppressor cells*. Hum Immunol 2002;63:1010-8.
- [3] National Government of Italy, Department of Health, Available from: <http://www.trapianti.salute.gov.it/> and http://www.trapianti.salute.gov.it/imgs/C_17_pubblicazioni_1511_allegato.pdf
- [4] Pretagostini R, Peritore D, Donadio R, et al. *Organizzazione Centro-Sud Trapianti: outcome analysis 1999 to 2002*. Transplant Proc 2004;36:439-41.
- [5] Gudex CM. *Health-related quality of life in endstage renal failure*. Qual Life Res 1995;4:359-66.
- [6] Voiculescu A, Ivens K, Hetzel GR, et al. *Kidney transplantation from related and unrelated living donors in a single German centre*. Nephrol Dial Transplant 2003;18:418-25.
- [7] Balaska A, Moustafellos P, Gourgiotis S, et al. *Changes in health-related quality of life in Greek adult patients 1 year after successful renal transplantation*. Exp Clin Transplant 2006;4:521-4.
- [8] Wong G, Howard K, Chapman JR, et al. *Comparative survival and economic benefits of deceased donor kidney transplantation and dialysis in people with varying ages and co-morbidities*. PLoS One 2012;7:e29591.
- [9] Cornella C, Brustia M, Lazzarich E, et al. *Quality of life in renal transplant patients over 60 years of age*. Transplant Proc 2008;40:1865-6.
- [10] Han SS, Sun HK, Lee JP, et al. *Outcome of renal allograft in patients with Henoch-Schönlein nephritis: single-center experience and systematic review*. Transplantation 2010;89:721-6.
- [11] Muehrer RJ, Becker BN. *Life after transplantation: new transitions in quality of life and psychological distress*. Semin Dial 2005;18:124-31.
- [12] Painter P, Krasnoff JB, Kuskowski M, et al. *Effects of modality change on health-related quality of life*. Hemodial Int 2012;16:377-86.
- [13] Látos M, Barabás K, Lázár G, et al. *Mental representations of the new organ and posttransplant patients' anxiety as related to kidney function*. Transplant Proc 2012;44:2143-6.
- [14] Lee AJ, Morgan CL, Conway P, et al. *Characterisation and comparison of health-related quality of life for patients with renal failure*. Curr Med Res Opin 2005;21:1777-83.
- [15] Callaghan CJ, Bradley JA. *Current status of renal transplantation*. Methods Mol Biol 2006;333:1-28.
- [16] Liem YS, Wong JB, Winkelmayer WC, et al. *Quantifying the benefit of early living-donor renal transplantation with a simulation model of the Dutch renal replacement therapy population*. Nephrol Dial Transplant. 2012;27:429-34.
- [17] Yildirim A. *The importance of patient satisfaction and health-related quality of life after renal transplantation*. Transplant Proc 2006;38:2831-4.
- [18] Ogutmen B, Yildirim A, Sever MS, et al. *Health-related quality of life after kidney transplantation in comparison intermittent hemodialysis, peritoneal dialysis, and normal controls*. Transplant Proc, 2006;38:419-21.

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A study on occupational exposure of Sicilian farmers to *Giardia* and *Cryptosporidium*

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Key words

Giardia/Cryptosporidium • Calves • Occupational risk

Summary

Introduction. A cross-sectional study was undertaken to determine the prevalence of *Giardia* and *Cryptosporidium* in calves of Palermo area (Sicily) and to evaluate the occupational risk associated with occurrence of zoonotic genotypes.

Methods. A total of 217 faecal samples, from 149 calves (between 2 and 240 days of age) and 68 farmers, were collected in 19 cattle-farms of Palermo area. A questionnaire regarding demographic characteristics and personal hygienic measures was submitted to all farmers. All faecal samples were analyzed by Immunofluorescence assay and Polymerase Chain Reaction (PCR); genotypes were determined by DNA sequencing of Triose Phosphate

Isomerase gene for *Giardia* and Small Subunit Ribosomal RNA gene for *Cryptosporidium*.

Results. None farmer tested was positive for *Giardia* and *Cryptosporidium*, whereas these protozoa were respectively detected in 53 (including 5 with zoonotic *G. duodenalis* genotype A) and 17 (of which 1 with zoonotic *C. ubiquitum*) of the examined calves.

Discussion. The results indicate that the risk of transmitting both protozoa to farmers in Palermo area is negligible although it cannot be considered null because of identification of human genotypes/species in calves.

Introduction

Giardia duodenalis (synonyms are *G. intestinalis* and *G. lamblia*) and *Cryptosporidium* spp. are common parasitic protozoa responsible for enteric illness in humans and animals worldwide [1]. *Cryptosporidium* in particular is a serious pathogen in immunocompromised individuals who may suffer from persistent life-threatening gastroenteritis [2]. The (oo)cysts are excreted in the faeces of infected hosts and transmitted through the faecal-oral route, contaminated water (drinking/recreational), ingestion of contaminated food and direct contact between humans and infected animals [3, 4]. Transmission via direct contact, in particular, is related to workers such as farmers and veterinarians and it suggests that compliance with hygienic standards (i.e. frequent hand-washing or constant use of gloves) to prevent transmission of both protozoa to persons handling animals. The infected hosts shed a lot of (oo)cysts in their faeces, for example one calf (≤ 30 days old) can produce up to 6×10^8 *Cryptosporidium* oocysts per gram of faeces, thereby increasing environmental contamination [5]. The (oo)cysts are robust and very resistant to disinfectants and can last for long periods (months) in the environment [6]. Sources of contamination of water and food may be different, but an important role is played by farm animals that act as reservoirs of infection. *Cryptosporidium* and *Giardia* are common in domestic livestock (cattle, sheep and pigs), where young animals can have a high prevalence of infection, shedding large numbers of (oo)cysts.

In recent years, the zoonotic potential of both protozoa is becoming clearer with the use of molecular techniques to genotype isolates. Molecular characterization of *G. duodenalis* has led to subdivision of the species into seven distinct assemblages (A, B, C, D, E, F, G): humans are infected with assemblages A and B which can also infect wildlife, companion animals and livestock; C and D, isolated from dogs; E, isolated from livestock; F, isolated from felines; and G, isolated from rats. Molecular analyses have also shown that the genus *Cryptosporidium* comprises 19 valid species and nearly twice as many genotypes [6]; *C. parvum* and *C. hominis* are the main species infecting humans [7]. Cattle are infected with at least five *Cryptosporidium* species (*C. parvum*, *C. andersoni*, *C. bovis*, *C. ryanae* and *C. suis*) and are considered the main reservoir of zoonotic *C. parvum* [8]. The distribution of *Cryptosporidium* species in dairy cattle is age-related: *C. parvum* is reported to primarily infect pre-weaned calves (5 days to 5 months) [9], *C. bovis* is found to predominate in 3 month to 2 year-old dairy cattle [8], *C. andersoni* usually infects mature cattle, resisting for years, if not for life. In Canada a prevalence in dairy calves (0-24 weeks of age) has been reported between 45.7 % and 73 % for *G. duodenalis*, and between 40.6 % and 88.7 % for *Cryptosporidium* spp., suggesting a potential risk of zoonotic transmission between cattle and farm workers [10]. In India, *G. duodenalis* assemblage A subgenotype 1 (A1), which is considered the most common zoonotic agent, was identified in both calves and workers, confirming that there is a

risk of occupational transmission of *G. duodenalis* infections between cattle and humans on dairy farms [11]. The strongest epidemiological evidence for zoonotic transmission of cryptosporidiosis is from investigations associating cattle with outbreaks in veterinary students handling infected young calves, animal researchers in contact with infected young calves, and children attending agricultural camps and fairs [12].

In a previous study carried out in Sicily [13], we demonstrated that the Oreto river which crosses the city of Palermo was contaminated by *Giardia* and *Cryptosporidium* and that *Giardia* was present in a high concentration in the water of the river. Our subsequent paper confirmed higher prevalence of *Giardia* than *Cryptosporidium* and showed that the Oreto river was contaminated by assemblages A and B of *Giardia*, with a predominance of zoonotic genotype A [14].

The aim of this study is to obtain data about prevalence of these two parasites in calves and farmers who come from cattle farms in the Palermo area, and to determine the occupational risk associated with occurrence of species and genotypes at zoonotic risk.

Methods

COLLECTION OF SPECIMENS

A list of commercial cattle farms was obtained from Veterinary District-Azienda Sanitaria Provinciale 6 (ASP 6) in Sicily. Between February 2009 and July 2010, 19 (19/62) farms from the eastern area of the city of Palermo were chosen due to the recent birth of calves, after which the farmers, who had been contacted by veterinarians of the ASP 6, confirmed their participation in the study. If any farm chose not to participate, it was excluded from the study and replaced by another farm. Selected farms included from 5 to 95 cows: four herds had from 5 to 10 animals, seven from 11 to 40, five from 41 to 70, three from 71 to 95; 11 farms were in the wild, the remaining 8 were cattle shed farms.

A total of 149 calves faecal samples were collected directly from the rectum of each animal using a disposable latex glove; 69 samples were collected during winter months and 80 during summer months. The calves, with or without diarrhoea, aged between 2 and 240 days of age (52 ranged from 2 to 60 days, 74 from 61 to 150 days, 23 from 151 to 240 days); 17 calves (7-150 days age) showed evidence of watery diarrhoea.

Human faecal samples were obtained from the faeces of 68 farmers (66 males and 2 females; the age ranged from 18 to 63 years) and examined for the presence of *Giardia* and *Cryptosporidium*. Simultaneously with the collection of samples, workers received a medical-social questionnaire comprising 15 questions.

All faecal samples were placed in a sterile vessel and transported to the laboratory in a proper recipient at 4° C for analysis within 24 hours of collection.

CONCENTRATION OF (OO)CYSTS FROM FAECES

The animal faecal samples were subjected to purification by a sucrose flotation technique as previously described [15]. Approximately 3 g of faeces were suspended in 10 mL of phosphate buffered saline (PBS). The suspension was filtered through a surgical gauze sponge and the filtrate was stratified into 5 ml of 1 M sucrose (Sigma-Aldrich) solution. The suspension was centrifuged at 800 x g for 5 min without brake. Following centrifugation, the interface and the upper layer of liquid was transferred to a clean tube and recentrifuged at 800 x g for 5 min. The supernatant was decanted and the pellet resuspended in PBS to a volume of 1 ml to examine by immunofluorescence assay.

All human faecal samples were examined by formol-ether concentration. Briefly, 2-3 g of faeces were suspended in 10 mL of saline solution and filtered through a surgical gauze. The filtrate was then centrifuged at 1500 rpm for 2 min. The supernatant was decanted and the pellet was suspended in 7 ml of formalin at 7% and 3 ml of ethyl acetate. The sample was mixed for 3 min and centrifuged at 1500 rpm for 2 min. The supernatant was discharged and the final pellet was examined by immunofluorescence assay (IFA).

IMMUNOFLUORESCENCE ASSAY (IFA)

All resulting pellet were processed by IFA (Merifluor *Cryptosporidium*/*Giardia* assay; Meridian Biosciences) for the simultaneous detection of *Cryptosporidium* and *Giardia*. The slides were observed with an epifluorescence microscope at 400 x magnification for the detection of FITC-mAb labeled oocysts/cysts. Presence of stained oocysts/cysts was identified according to morphology.

MOLECULAR ANALYSIS

The IFA positive *Giardia* and *Cryptosporidium* samples were subjected to molecular analysis. DNA was extracted as described by da Silva et al. [16]. The PCR of *Giardia* and *Cryptosporidium* were performed by amplification of the triose phosphate isomerase (TPI) and small subunit ribosomal RNA (ssuRNA) genes, respectively. Nested-PCR was used to amplify fragments of both genes [17]. The PCR products were analyzed by 1% agarose gel electrophoresis and visualized after ethidium bromide staining.

The secondary PCR products were purified using Microcon PCR centrifugal filter devices (Millipore Corp., Bedford, MA) and sequenced on an ABI 3100 automated sequencer (Perkin Elmer). Sequence accuracy was confirmed by sequencing an independent PCR product on each strand. Multiple alignments were performed using the computer software package Clustal X [18].

Published *Giardia* TPI and *Cryptosporidium* ssuRNA nucleotide sequences were aligned with the homologous sequences determined in the present work.

QUESTIONNAIRE

During this study 74 farmers were willing to participate and were enrolled into the survey; six subjects refused to answer the questionnaire and were therefore excluded from the study. All enrolled participants (n=68) declared not to be in an immunosuppressed status.

A series of multiple choice questions (n = 15) were asked regarding the attitude and practices towards preventive measures taken to avoid occupational infections as well as socio-demographic characteristics (sex, age, length of service, distance between farm and home, household composition) and medical information (diarrhoea events) were also collected. Subjects were asked the circumstances of hand washing (i.e. during handling animals, at the beginning or end of the working day) and the use of gloves, the custom of biting one's nails and of changing clothing before going home. Questions about changing clothes and shoes were used to understand infectious risk levels for themselves and for their families as the clothing, and in particular the shoes, could be carriers of *Giardia* and *Cryptosporidium* (oo)cysts and therefore play a role in their transmission to other environments. Moreover, the participants were asked to answer two questions related to a number of personal cases of diarrhoea during the last year and to those that occurred in their families during the last 6 months.

Statistical analysis

All collected data were entered into an electronic database and analyzed using Epi Info software, version 7. Calves age was categorized into three groups (2 to 60 days; 61 to 150 days and 151 to 240 days). Absolute and relative frequencies were calculated for qualitative variables, while quantitative non-normally distributed variables were summarized as median and/or range. Normal distribution was verified by the Shapiro-Wilk test for normality.

Results

Assemblages/Species of *Giardia* and *Cryptosporidium*

Giardia and *Cryptosporidium* were found in 78.9% and in 52.6% of the cattle farms, respectively. The overall proportion of animal samples containing detectable *G. duodenalis* cysts and *Cryptosporidium* oocysts was 35.6 % and 11.4 %, respectively. Mixed infections of *Giardia* and *Cryptosporidium* were found in 14 dairy calves out of 149; of these, 10 were detected in animals from 2 to 60 days of age, 2 in those between 61-150 days of age and 2 between 180-240 days of age. Moreover, we observed no seasonality among the *Giardia* and *Cryptosporidium* infections, instead as previously reported in Norway [19] where the prevalence of both parasites was higher in samples taken during winter than in samples taken during summer.

There was a large variation in prevalence of *Giardia* and *Cryptosporidium* infected animals among age groups. Only for *Giardia* there was a trend for decreasing intensity of infection with increasing age of calves. In fact, prevalence of *Giardia* varied from 65.4 % to 20.2 % and 17.4 %, while for *Cryptosporidium* it varied from 21.1 % to 2.7 % and 17.4 % (Tab. I). Faecal consistency at sampling was watery in 14 younger calves (under 60 days) and in 3 animals of age-group 61-150 days; none of the 23 calves older than 150 days showed diarrhoea. The watery consistency of the stools was associated with increased detection of both parasites. *Giardia* was detected in 16 while *Cryptosporidium* in 6 of 17 diarrhoeic faecal samples (Tab. I).

All 68 human faecal samples were negative for detection of *G. duodenalis* and *Cryptosporidium* spp. including farmers (n = 15) who had contact with infected animals and did not wash their hands at work.

Of the animal samples tested positive for *Giardia* and *Cryptosporidium* by IFA, the PCR was successful in 34 (25 ranged from 2 to 60 days, 8 from 61 to 150 days, 1 from 151 to 240 days) and 12 (10 ranged from 2 to 60

Tab. I. Age-related prevalence of *Giardia* and *Cryptosporidium* in 149 calves between 2 and 240 days of age, coming from 19 farms, as determined by IFA.

Calves age	Total calves	Faecal consistency	<i>Giardia</i> Positive IFA (%)	<i>Cryptosporidium</i> Positive IFA (%)	Mixed infections (<i>Giardia/Cryptosporidium</i>) (%)
2-60 days	52	14 watery 38 normal	14 20 (65.4)	5 6 (21.1)	5 5 (10.9)
61-150 days	74	3 watery 71 normal	2 13 (20.2)	1 1 (2.7)	2 0 (2.7)
151-240 days	23	0 watery 23 normal	0 4 (17.4)	0 4 (17.4)	0 2 (8.1)
	Tot 149		(35.6)	(11.4)	(9.4)

Tab. II. Age-related positive samples for *Giardia* and *Cryptosporidium* by PCR and identification of genotypes as determined by sequencing of the TPI gene for *Giardia* and SSUrRNA gene for *Cryptosporidium*.

Calves age	<i>Giardia</i>	<i>G. duodenalis</i> genotypes (n)	<i>Cryptosporidium</i>	<i>Cryptosporidium</i> species (n)
	No. of positive samples by PCR/No. of positive samples by IFA		No. of positive samples by PCR/No. of positive specimens by IFA	
2-60 days	25/34	A (4); E (15) ^a	10/10	<i>C. ryanae</i> (4) <i>C. bovis</i> (5) <i>C. ubiquitum</i> (1)
61-150 days	8/15	A (1); E (6) ^b	1/1	<i>C. bovis</i> (1)
151-240 days	1/4	E (1)	1/1	<i>C. bovis</i> (1)

^a 19/25 PCR products were successfully sequenced

^b 7/8 PCR products were successfully sequenced

days, 1 from 61 to 150 days, 1 from 151 to 240 days) samples respectively. *Giardia* genetic sequencing was successful on 27 of the 34 PCR positive samples.

Giardia genotypes showed 100% homology with assemblage E (n = 22) (GenBank accession number AB569406), and assemblage A (n = 5) (GenBank accession number AB569398); the zoonotic genotype *Giardia* assemblage B was not detected.

Of the 12 samples from which DNA amplification and genetic sequencing was obtained for *Cryptosporidium*, 7 samples exhibited 100% homology with *C. bovis* (GenBank accession number HQ179575), 4 samples had 100% homology with *C. ryanae* (GenBank accession number HQ179574), and finally 1 sample showed 100% homology with *C. ubiquitum* (GenBank accession number EU827382) (Tab. II).

QUESTIONNAIRE RESPONSES

68/74 farmers returned the completed questionnaire. The analysis of the demographic and practice characteristics of the study group showed that the majority was male (n = 66); the age of respondents ranged from 18 to 63 years, with a median age of 35 years. 38 respondents had worked for more than 15 years, while 20 had worked for 4 to 15 years and 10 for less than 4 years. The household composition of 49 participants ranged from 3 to 5 members: of 14 participants from 6 to 7 and finally of 5 participants from 1 to 2. The distance between farm and home is more than 1 kilometre for 58 farmers, while 10 people live next to the farm. The behaviours related to use of protective equipment and to personal hygiene and their clinical status were also evaluated as shown in Table III.

Discussion

To our knowledge, this is the first study to report prevalence and molecular characterization data of *G. duodenalis* and *Cryptosporidium* spp. in cattle farms in the Palermo area. Data collected from 19 farms in Palermo area indicate that giardiasis and cryptosporidiosis are

common infections of calves and that the prevalence of *Giardia* infection is higher than that of *Cryptosporidium*. This result is in agreement with the previously reported findings in Spain [20] and in Australia [21], but not with those described in Portugal, where the prevalence of *Cryptosporidium* exceeded that of *Giardia* [22]. Moreover, ours and other data in literature report two main points: a) the decreasing age-related prevalence of *Giardia* in calves, b) the concurrent *G. duodenalis* and *Cryptosporidium* spp. infections [23].

We have found two distinct genotypes of *G. duodenalis*: assemblage A, which is also isolated from humans and other animals and proposed to be zoonotic [23], and assemblage E, which is believed to be specific for livestock [24]. However, recent studies conducted in Uganda and in Egypt revealed the presence of assemblage E in non-human primates (red colobus monkeys) and in humans respectively [25, 26]. Assemblage E has been reported in the area of Gharbia (Egypt) where two thirds of the inhabitants belong to a rural community who live in close contact with their livestock and implement inadequate hygiene practices. However, because of a high prevalence of assemblage E in calves [25] and of the possible cattle-primate transmission link, a strong implementation for future research into the epidemiology, cross-species transmission ecology and clinical consequences of *G. duodenalis* infection in humans are required. In this study it is probable that both the lack of the above mentioned inadequate conditions of hygiene in our area may be cause for the no transmission of zoonotic and non-zoonotic genotypes/species to farmers.

In particular, in the present study there are three main hygienic measures implemented by the participants: 1) no biting of nails, 2) change of shoes before returning home, 3) hand-washing prior to eating, drinking, or smoking at work and after contact with animals. Nails, in particular long nails, are a receptacle of microorganisms and the absence of the practice of nail-biting (82.4% of respondents) is a healthy measure that can prevent infections in individuals handling infected animals. Changing shoes at the end of the day (36.7% of respondents) is an important hygienic measure because

Tab. III. Compliance with hygienic measures and reports of diarrhoea in 68 farmers who responded to the survey.

Personal hygienic measures	Frequency	N	%
Use of gloves at work	always	12	17.6
	sometimes	18	26.5
	never	38	55.9
Washing hands before drinking/eating/ smoking at work	always	37	54.4
	sometimes	16	23.5
	never	15	22.1
Washing hands after animal contacts	always	40	58.8
	sometimes	3	4.4
	never	25	36.8
Washing hands at the end of working day	always	13	19.1
	sometimes	14	20.6
	never	41	60.3
Washing hands return home	always	25	36.8
	sometimes	10	14.7
	never	33	48.5
Practice of nail biting	always	10	14.7
	sometimes	2	2.9
	never	56	82.4
Change of work clothes at the end of the day	always	25	36.7
	sometimes	31	45.6
	never	12	17.7
Change of work shoes at the end of the day	always	43	63.2
	sometimes	13	19.1
	never	12	17.7
Diarrhoea during last year	no episode	49	72.0
	1 episode	8	11.8
	2 episodes	7	10.3
	> 2 episodes	4	5.9
Diarrhoea in family during last 6 months	no episode	62	91.2
	1 episode	6	8.8
	2 episodes	0	0
	> 2 episodes	0	0

the soles could be carriers of *Giardia* and *Cryptosporidium* (oo)cysts (present in soil) which then spread at home, with consequent risks of infection among family members, especially children who often play on the ground. Hands represent the principal route of transmission (i.e. person-to-person or animal-to-person) of many pathogens that can survive for a long time on the skin, in particular *Giardia* and *Cryptosporidium* that produce robust (oo)cysts, therefore a high compliance with hand-washing is of crucial importance to prevent the transmission of microorganisms that can be transmitted orally when hands are brought near the mouth to eat, drink or smoke. However, it is important to emphasize that other infectious occupational risks (i.e. mycosis) [27] associated with animal contact highlight a need for the use of personal protective equipment, and the infrequent use of gloves in this report is a critical point that identifies farmers of the Palermo area as a target group for future educational campaigns of public health, as educational interventions may improve knowledge and practice related to prevention.

Furthermore, we have not found either the zoonotic *Giardia* assemblage B (which has only occasionally been reported in calves), nor the zoonotic *C. parvum* found up to 100% of calves at 1 and 2 weeks of age [9]. On the contrary, *C. bovis* (considered cattle-specific) was

the most common species identified (n=7), particularly in age-group 2-60 days (n = 5), as already reported in China, India, Georgia and western North Dakota [28-30]. Recently, *C. bovis* has been identified in farm workers in rural areas both in India [11] and in Australia [31], providing evidence of zoonotic transmission and the possible association of infected calves and human infection with *Cryptosporidium*; interestingly, in both studies no clinical symptom was reported from infected individuals.

Conclusion

The absence of *C. parvum*, a major pathogen in humans, the occurrence only once of *C. ubiquitum*, potentially zoonotic, the relative low prevalence of *C. bovis* as well as the small number (n = 5) of zoonotic *G. duodenalis* assemblage A, suggest that calves may not be significant in the epidemiology of human giardiasis and cryptosporidiosis in the Palermo area.

For *Giardia* and *Cryptosporidium* there is a need for molecular epidemiological studies to be undertaken in well-defined foci of transmission in order to fully determine the frequency and importance of zoonotic transmission, in particular because cryptosporidiosis affects both immunocompetent and immunocompromised individuals

and in these last subjects is responsible of severe life-threatening illness [2]. Although the risk of transmitting *Giardia* and *Cryptosporidium* to occupationally exposed subjects in farms in Palermo area is negligible due to the low prevalence of protozoa and biosecurity measures implemented, the obtained data suggest that calves

can act as a source of giardiasis and cryptosporidiosis for farm workers and responsible of environmental contamination. We believe that more studies involving extensive sampling of both calves and farmers are necessary in Palermo area and in other geographic areas of Sicily.

References

- [1] Smith HV, Cacciò SM, Cook N, et al. *Cryptosporidium* and *Giardia* as foodborne zoonoses. *Vet Parasitol* 2007;149:29-40.
- [2] Del Coco VF, Córdoba MA, Basualdo JA. *Cryptosporidiosis: an emerging zoonosis*. *Rev Argent Microbiol* 2009;41:185-96.
- [3] Chappell CL, Okhuysen PC. *Cryptosporidiosis*. *Curr Opin Infect Dis* 2002;15:523-7.
- [4] Ali SA, Hill DR. *Giardia intestinalis*. *Curr Opin Infect Dis* 2003;16:453-60.
- [5] Uga S, Matsuo J, Kono E, et al. *Prevalence of Cryptosporidium parvum infection and pattern of oocyst shedding in calves in Japan*. *Vet Parasitol* 2000;94:27-32.
- [6] Fayer R. *Taxonomy and species delimitation in Cryptosporidium*. *Exp Parasitol* 2010;124:90-7.
- [7] Xiao L. *Molecular epidemiology of cryptosporidiosis: an update*. *Exp Parasitol* 2010; doi: 10.1016/j.exppara.2009.03.018.
- [8] Fayer R, Santin M, Trout JM, Greiner E. *Prevalence of specie and genotypes of Cryptosporidium found in 1 to 2-year-old dairy cattle in the eastern United States*. *Vet Parasitol* 2006;135:105-12.
- [9] Santin M, Trout JM, Xiao L, et al. *Prevalence and age-related variation of Cryptosporidium species and genotypes in dairy calves*. *Vet Parasitol* 2004;122:103-17.
- [10] Coklin T, Farber J, Parrington L, et al. *Prevalence and molecular characterization of Giardia duodenalis and Cryptosporidium spp. in dairy cattle in Ontario, Canada*. *Vet Parasitol* 2007;150:297-305.
- [11] Khan SM, Debnath C, Pramanik AK, et al. *Molecular characterization and assessment of zoonotic transmission of Cryptosporidium from dairy cattle in West Bengal, India*. *Vet Parasitol* 2010; doi: 10.1016/j.vetpar.2010.03.008.
- [12] Xiao L, Fayer R. *Molecular characterization of species and genotypes of Cryptosporidium and Giardia and assessment of zoonotic transmission*. *Int J Parasitol* 2008; doi: 10.1016/j.ijpara.2008.03.006.
- [13] Di Benedetto MA, Di Piazza F, Maida CM, et al. *Occurrence of Giardia and Cryptosporidium in wastewater, surface water and ground water samples in Palermo (Sicily)*. *Ann Ig* 2005;17:367-75.
- [14] Di Benedetto MA, Di Piazza F, Maida CM, et al. *Molecular characterization of Giardia duodenalis cysts in the Oreto river (Sicily, Southern Italy)*. *Ital J Public Health* 2010;7:62-8.
- [15] Olson ME, Guselle NJ, O'Handley RM, et al. *Giardia and Cryptosporidium in dairy calves in British Columbia*. *Can Vet J* 1997;38:703-6.
- [16] da Silva AJ, Bornay-Llinares FJ, Moura INS, et al. *Fast and reliable extraction of protozoan parasite DNA from faecal specimens*. *Mol Diagn* 1999;4:57-64.
- [17] Sulaiman IM, Ronald F, Bern C, et al. *Triosephosphate isomerase gene characterization and potential zoonotic transmission of multispecies Giardia duodenalis*. *Emerg Infect Dis* 2003;9:1444-52.
- [18] Thompson JD, Gibson TJ, Plewniak F, et al. *The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools*. *Nucleic Acids Res* 1997;24:4876-82.
- [19] Hamnes IS, Gjerde B, Robertson L. *Prevalence of Giardia and Cryptosporidium in dairy calves in three areas of Norway*. *Vet Parasitol* 2006;140:204-16.
- [20] Castro-Hermida JA, Carro-Corral C, González-Warleta M, et al. *Prevalence and intensity of infection of Cryptosporidium spp. and Giardia duodenalis in dairy cattle in Galicia (NW Spain)*. *J Vet Med B Infect Dis Vet Public Health* 2006;53:244-6.
- [21] Yang R, Mc Carthy S, Gordon C, et al. *Molecular characterization of Cryptosporidium and Giardia in pre-weaned calves in Western Australia and New South Wales*. *Vet Parasitol* 2011;176:145-50.
- [22] Mendonca C, Almeida A, Castro A, et al. *Molecular characterization of Cryptosporidium and Giardia isolates from cattle from Portugal*. *Vet Parasitol* 2007;147:47-50.
- [23] Thompson RCA. *Giardiasis as a re-emerging infectious disease and its zoonotic potential*. *Int J Parasitol* 2000;30:1259-67.
- [24] Thompson RC. *The zoonotic significance and molecular epidemiology of Giardia and giardiasis*. *Vet Parasitol* 2004;126:15-35.
- [25] Johnston AR, Gillespie TR, Rwego IB, et al. *Molecular epidemiology of cross-species Giardia duodenalis transmission in western Uganda*. *PLoS Negl Trop Dis* 2010; doi: 10.1371/journal.pntd.0000683.
- [26] Foronda P, Bargues MD, Abreu-Acosta N, et al. *Identification of genotypes of Giardia intestinalis of human isolates in Egypt*. *Parasitol Res* 2008;103:1177-81.
- [27] Sahin I, Kaya D, Parlak AH, et al. *Dermatophytoses in forestry workers and farmers*. *Mycoses* 2005;48:260-4.
- [28] Feng Y, Ortega Y, He G, et al. *Wide geographic distribution of Cryptosporidium bovis and the deer-like genotype in bovines*. *Vet Parasitol* 2007;144:1-9.
- [29] Barigye R, Dyer NW, Newell TK, et al. *Molecular and immunohistochemical detection of assemblage E, Giardia duodenalis in scouring North Dakota calves*. *Vet Parasitol* 2008;157:196-202.
- [30] Feltus DC, Giddings CW, Khaita ML, et al. *High prevalence of Cryptosporidium bovis and deer-like genotype in calves compared to mature cows in beef cow-calf operations*. *Vet Parasitol* 2008;151:191-5.
- [31] Ng JS, Eastwood K, Walker B, et al. *Evidence of Cryptosporidium transmission between cattle and humans in northern New South Wales*. *Exp Parasitol* 2012; doi: 10.1016/j.exppara.2012.01.014.

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Psychosocial predictors of barriers to cervical cancer screening among Iranian women: the role of attachment style and social demographic factors

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Key words

Attachment • Screening barriers • Cervical cancer

Summary

Objectives. Despite advances in screening and treatment during past several Decades, cervical cancer remains a major health problem for Iranian women. Recent researches have focused on factors related to development of health behavior in an effort to design effective early interventions. The current study aimed to investigate the role of attachment styles on cervix cancer screening barriers among women of BandarAbbas-Iran.

Methods. In an analytic-cross sectional study, 681 women aged 21-65 referring to health centers were selected randomly and after completing written informed consents were investigated by Revised Adult Attachment Scale (RAAS) (Collins and Read), Pap smear screening barriers and demographic data questionnaire. The data were analyzed by Pearson correlation coefficient, linear regressions and chi-square test.

Results. The results showed significant association between attachment styles and screening barriers. There was a negative significant relation between secure attachment style and screening barriers and there was a positive significant association between insecure attachment style (anxiety and avoidant) and screening barriers. The regression analysis indicated that insecure attachment style (avoidant) were predictors of barriers to the Pap smear screening test in this regard. There was a significant association between age and residential area and participation in Pap smear test.

Conclusions. Insecure attachment style is associated with hazardous risk behaviors and these results can be useful for health service providers in preventive planning of screening and identification of people susceptible to risk and the design of the intervention.

Introduction

Cervical cancer is a global health problem (Population Reference Bureau and Alliance for Cervical Cancer Screening, 2004). According to the World Health Organization (2009) cervical cancer is the second biggest cause of female cancer mortality worldwide. Not only would cervical cancer kill 288000 women every year, but 510000 would be diagnosed annually. Nearly 80% of women suffering from cervical cancer live in developing countries [1]. According to the cancer registration report, this cancer is reported about 7.1% showing the importance of cervix cancer among women cancer in Iran [2]. Invasive cervix cancer can be prevented because it has a long premalignant stage, second the treatment of pre-invasive lesion is effective and third screening cytology of cervix cancer including Pap smear are available to the public [3]. The proposed protocol for cervix cancer screening by USA cancer association in 2012 was performing Pap smear each 3 years among women aged 21-65 year old. This experiment should be done each 1-3 year among the women being active sexually [4]. Now, screening and cervix cytology (Pap smear) is one of the most effective and cheapest methods for cervix

cancer screening [5]. According to the high prevalence of cervix cancer among women, high diagnosis of Pap smear test and its easy application, it is expected that all women refer for regular test. Unfortunately, despite Pap smear test, the mortality rate of cervix cancer is not reduced considerably in Iran. Thus, the identification of the factors leading into the participation or non-participation in health-related behaviors can lead into the reduction of health weak behaviors. In the previous studies, some important variables of these decisions are identified. One of the variables is socio-economic variables: The people with better education and the people with the parents of high socio-economical conditions are more inclined to participate in healthy behaviors [6, 7]. In various researches, the role of psychological factors on health preventive behaviors is shown. The attachment styles are of great importance due to the important role in interpersonal relations and the reaction of a person in stressful situations [8-13]. Attachment is a stable emotional relation and its characteristic is tendency to search and keeping closeness with a person, namely in stressful situations [14, 15]. According to Bowlby (1969), the people internalize the initial experiences with the care providers and form stable cognitive psychological

structures of the relations (Schema). These structures affect the image of a person of himself (self model) as care qualified person and deserving the aid and support of other (other model). The negative model of the self means that he is not entitled to care and is related to anxiety in interpersonal relations while the negative model of the other is defined by the lack of confidence of others and public avoidance in the relations. These cognitive structures or internal working models affect a person interaction with others and his interpretation of them during the life [16, 17]. Bowlby (1973, 1969) cited in Batman and Jaspersen (2008) believed that during the stress, people rely on attachment relations by keeping the closeness with a person helping the helplessness management and it creates comfort. He believed that attachment is activated during the threat. In other words, disease is the threat, isolation and attachment activates attachment behavior. The adult patients show their attachment styles in therapy relations. Bartholomew believed that personal differences in attachment styles are applied in interpersonal relations including the relation between the patient and the caregiver. Therefore, attachment theory is a useful model to recognize the perception of the signs, medical care and interaction with the care givers [18]. The recent research about attachment is dedicated to the application of attachment theory in medical diseases. The attachment theory based on the fundamental assumptions can integrate the biological, mental and social variables and health and disease to determine the consistency with physiological disease [8]. The researches showed that insecure attachment style is related with the development of health problems via low participation in health protective behaviors [11, 19]. The researches showed that the people with insecure attachment style compared to the people with secure attachment styles little perform the health- related behaviors (e.g. sport, diet, stopping alcohol and smoking) [20]. In addition, Scarf et al. (2001), showed positive and significant relation between secure attachment style and health protective behaviors [21]. There is no study on the effect of socio-psychological factors on the cervix cancer screening barriers in Iran, the current study conducted with the aim of investigating the effect of attachment style on barriers of Pap smear screening test among women in BandarAbbas-Iran to provide background information for educational intervention of screening program and women education.

Methods

SAMPLE

The current analytic cross-sectional study conducted in 2011-2012 in BandarAbbas-Iran. The study population was all married women aged 20-65 referring to health centers of BandarAbbas-Iran. The inclusion criteria were as follow: Marriage, referring to the clinic to receive one of the services (pregnancy control, postnatal care, family control consulting, women disease, children vaccination). The exclusion criteria were as follow: Genital

cancer history or lack of tendency to participate in the study. According to the mentioned criteria, 681 women were selected by simple random sampling. For ethical issues, before the completion of the questionnaires, the participants completed written informed consent and were assured that their information will be confidential.

INSTRUMENTS AND MEASURES

1) Revised Adult Attachment Scale (Collins, 1996; Collins & Read, 1990).

The Revised Adult Attachment Scale (RAAS) (Collins & Read, 1990) is an 18-item measure of adult attachment dimensions. It consists of three subscales: Close, Depend and Anxiety. The Close subscale measures the level of comfort the individual feels with closeness and intimacy. The Depend subscale assesses if the individual feels they can depend on others to be available when needed. The Anxiety subscale measures the level of anxiety the person feels about being rejected or unloved. High scores on Close and Depend, and low scores on the Anxiety dimension, indicate a secure attachment style [22, 23]. Each item is scored on a 5-point Likert scale with some items being reverse scored. The RAAS has demonstrated adequate validity and reliability [22]. In the present investigation, the Cronbach alphas were .73 for Anxiety, .63 for Depend and .51 for Close, respectively.

2) Screening barriers questionnaire

The questionnaire of screening barriers of Pap smear is an 11-item questionnaire being designed for the evaluation of the barriers of Pap smear test. The items of the questions were derived from the questionnaire used to assess Pap test barriers by Hill and Gick [24]. Participants were asked to rate the 11 items, each regarding a potential barrier to cervical screening (e.g. too time-consuming, embarrassing), on a 7-point Likert scale ranging from "strongly disagree" to "strongly agree." A total Pap test barriers score was calculated by determining the mean Likert scale response. In the present study, the scale had excellent internal reliability ($\alpha = .83$). In data analysis, Pearson correlation coefficient, chi-square test and liner regression.

Results

In the present study 681 participants with age mean 33.96 completed the questionnaires by self-report method. 45.37% of them had academic education, 34.37% diploma and 20.26% had less than diploma degree. 60.64% of them were housewives and 39.36% were employed. Totally, 342 people (50.23%) had the history of Pap smear test.

Table I displays, secure attachment style had significantly negative relation with screening barriers and avoidant attachment style had positively significant relation with screening barriers. $P < 0.01$.

$F(3,680) = 11.13$ statistics imply regression equation to predict screening barriers based on attachment styles

Tab. I. Descriptive statistics and correlations of Attachment factors and screening barriers.

4	3	2	1	Variables
			1	1.Barriers
		1	-0.092**	Secure style.2
	1	0.331**	-0.025	Anxious style.3
1	-0.095**	-0.216**	0.211**	4.Avoidant style
15.64	17.27	20.56	36.48	Mean
4.49	4.20	3.63	14.34	SD

* 0.05 < p < 0.01 ** P

was significant ($p = 0.001$). Table II displays, avoidant attachment style is a significant predictive for screening barriers of Pap smear ($p = 0.001$).

Based on Table III, participation in Pap smear test was dependent upon the age of the participants ($P = 0.001$), the participation of the women was increased to its highest (58.8%) in 30-39 years and then decreased. The Participation in Pap smear test was dependent upon the residential area of the participants ($p = 0.019$). But not dependent on their job and education.

Discussion

The main aim of this study was to investigation of the role of attachment styles on screening barriers of cervix cancer. The results of the correlation between the varia-

bles showed that there was a significant negative relation between secure attachment style and screening barriers and there was a positive significant relation between insecure attachment styles (avoidant) with screening barriers. Attachment avoidance was the positive predictor for cervical screening barriers this result is in accordance to findings of other studies [25]. Among previous studies evaluating avoidant attachment style and its association with these relationship-oriented behaviors, the most consistent finding related to an association between the avoidant dimension of attachment and sexual behavior was an association with variables reflecting having had risky or casual partners [26, 27]. Both attachment dimensions have been linked to risky sexual behaviors such as increased sexual activity and a young age at first intercourse [27, 28]. This in turn may increase the risk of contracting the human papillomavirus (HPV), the sexually transmitted virus that causes cervical cancer [29]. Therefore, because of its association with cervical cancer risk-related behaviors (i.e. lack of screening and sexual activity; Health Canada, 2002), attachment insecurity may in turn be a risk factor for the development of cervical cancer. these results in combination with those from previous studies suggest that attachment style may be a useful framework for anticipating risk factors for a variety of common contributors to morbidity and mortality among adult women in this setting [30, 31]. Specially, an understanding of the influence of attachment style could be used to augment existing screening practices and allow practitioners or other team members (e.g., social workers, case managers, or therapists) to more quickly identify relationship-based risk factors for disease among persons with high levels of anxious attachment (e.g. sexual behaviors that place them at higher risk of STIs or unwanted pregnancy). It could also be used to help providers recognize when patients with high levels of avoidant attachment are using unhealthy strategies such as smoking to regulate negative emotions and/or are non-compliant with preventive/self care recommendations (e.g., when they are not using seatbelts or adhering to chronic disease treatment guide lines) [11, 32]. Maun-der and Hunter [11, 19] proposed that attachment insecurity is a risk factor for inadequate participation in health-protective behaviors, but the mechanisms underlying this relationship

Tab. II. Linear regression of attachment dimensions on Pap test barriers.

p	t	R ²	R	β	predictor variable
0.202	-1.278	0.04	0.21	-0.052	Secure
0.783	0.275			0.011	Attachment Anxious
0.001	5.236			0.201	Attachment Avoidant

Tab. III. The relation between demographic characteristics and Participating in Pap smear test among women.

p-value	Non Participated (n=339)	Participated (n=342)	Variable
0.061	11(73.3%) 28(62.2%) 44(56.4%) 111(74.4%) 145(46.9%)	4(26.7%) 17(37.8%) 34(43.6%) 123(52.6%) 164(53.1%)	Education level Illiterate Elementary Guidance school Diploma University
0.433	128(47.8%) 211(51.1%)	140(52.2%) 202(48.9%)	Job Employee Unemployed
0.019**	301(48.2%) 13(68.4%) 25(67.6%)	324(51.8%) 6(31.6%) 121(32.4%)	Residence City City zone Village
0.001**	155(60.5%) 110(41.2%) 48(45.3%) 26(50.0%)	101(39.5%) 157(58.8%) 58(54.7%) 26(50.0%)	Age 21-29 30-39 40-49 50-59

Data were presented as frequencies .(%) p-values marked with** were performed using Pearson Chi-square test.

remain speculative [11-14]. One possibility is that because attachment security is associated with trust in others and suitable help-seeking behaviors [34]. Individuals who are securely attached also seek health care when appropriate. Additionally, attachment security has been linked to the ability to employ effective coping mechanisms [34,35], which in turn could also be applied to cervical screening. Compared to those who are insecurely attached, securely attached individuals are more likely to perceive threats as challenges [36] and, in turn, effectively cope with such threats. It is possible that cervical screening may be perceived as a threat because of the stressful aspects of screening (e.g. the discomfort of the exam and waiting for test results), and therefore, attachment insecurity could be a hindrance in coping with these stressors. In order to clarify the association between attachment and cervical screening, future research should consider exploring potential moderators and mediators of the association, such as coping mechanisms and threat appraisals. Other findings of the study are the significant association between the age and residential area by doing Pap smear test. Similar to the findings of other studies, the participation of the women was increased to its high-

est (58.8%) in 30-39 years and then decreased [37-39]. The relationship between screening for cervical cancer and age, high educational level, and residential area suggest that these groups are more likely to participate in cervical cancer screening and this is consistent with previous studies [37, 40]. This study had a number of limitations. First conclusions from self-report data also demand caution. It is possible that the subjects gave non-exact responses in some cases. In addition, the cross-sectional study design precluded assessment of temporal relationship among variables. Finally selecting participants from health centers decreased the potential of generalizing the results to other populations. The results of the study are applied in the development of the interventions via considering the processes predicting the screening behaviors.

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References

- [1] Maree JE, Wright SCD. *Cervical cancer: does our message promote screening? A pilot study in a South African context.* European Journal of Oncology Nursing 2011;15:118-23.
- [2] Center for Disease Control Non Communicable Deputy Cancer Control Office. *Iranian Annual of National Cancer Registration Report.* Tehran: Ministry of Health and Medical Education 2008:26-34.
- [3] Fakour F, Atrkar Roshan Z, Shaef SP, et al. *Sensitivity and features of visual inspection of cervix with acetic acid, as a screening test for cervical cancer.* Journal of Guilan University of Medical Sciences 2011;79:49-55.
- [4] Saslow D, Solomon D, Lawson HW, et al. *American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer.* CA Cancer J Clin 2012;62:147-72.
- [5] Wright TC, Cox JT, Massad LS, et al. *Guidelines for the management of women with cervical cytological abnormality.* JAMA J Am Med Assoc 2004;287:2120-9.
- [6] Leganger A, Kraft P. *Control constructs: do they mediate the relation between educational attainment and health behaviour?* Journal of Health Psychology 2003;8:361-72.
- [7] Huurre T, Aro H, Rahkonen H. *Well-being and health behavior by parental socioeconomic status: a follow-up study of adolescents aged 16 until age 32 years.* Soc Psychiatry Psychiatr Epidemiol 2003;38:249-55.
- [8] Ciechanowski PS, Walker EA, Katon WJ, et al. *Attachment theory: A model for health care utilization and somatization.* Psychosom Med 2002;64:660-7.
- [9] Feeney JA. *Adult attachment, coping style and health locus of control as predictors of health behavior.* Aust J Psychol 1995;47:171-7.
- [10] Hunter JJ, Maunder RG. *Using attachment theory to understand illness behavior.* Gen Hosp Psychiatry 2001;23:177-82.
- [11] Maunder RG, Hunter JJ. *Attachment and psychosomatic medicine: Developmental contributions to stress and disease.* Psychosom Med 2001;63:556-67.
- [12] Maunder RG, Lancee WJ, Nolan RP, et al. *The relationship of attachment insecurity to subjective stress and autonomic function during standardized acute stress in healthy adults.* J Psychosom Res 2006;60:283-90.
- [13] Taylor RE, Mann AH, White NJ, et al. *Attachment style in patients with unexplained physical complaints.* Psychol Med. 2000;30:931-41.
- [14] Berry K, Barrowclough C, Wearden A. *A review of the role of adult attachment style in psychosis: unexplored issues and questions for further research.* Clin Psychol Rev 2007;27:458-75.
- [15] Platts H, Tyson M, Mason O. *Adult attachment style and core beliefs: are they linked?* Clin Psychol Psychother 2002;9:332-48.
- [16] Pickering L, Simpson J, Bentall RP. *Insecure attachment predicts proneness to paranoia but not hallucinations.* Pers Individ Dif 2008;44:1212-24.
- [17] Ciechanowski P, Sullivan M, Jensen M, et al. *The relationship of attachment style to depression, catastrophizing and health care utilization in patients with chronic pain.* Pain 2003;104:627-37.
- [18] Bettman EJ, Jaspersen RA. *Adults in wilderness treatment: a unique application of attachment theory and research.* Clinical Social Work Journal 2008;36:51-61.
- [19] Maunder RG, Hunter JJ. *Attachment relationships as determinants of physical health.* J Am Acad Psychoanal Dyn Psychiatry 2008;36:11-32.
- [20] Huntsinger ET, Luecken LJ. *Attachment relationships and health behavior: The meditational role of self-esteem.* Psychology & Health 2004;19:515-26.
- [21] Scharfe E, Eldredge D. *Associations between attachment representations and health behaviors in late adolescence.* J Health Psychol 2001;6:295-307.
- [22] Collins NL, Read SJ. *Adult attachment, working models, and relationship quality in dating couples.* J Pers Soc Psychol 1990;58:644-63.
- [23] Collins NL. *Working models of attachment: implications for explanation, emotion, and behavior.* J Pers Soc Psychol 1996;71:810-32.

- [24] Hill EM, Gick ML. *The big five and cervical screening barriers: Evidence for the influence of conscientiousness, extraversion and openness*. *Pers Individ Dif* 2011;50:662-7.
- [25] Hill EM, Gick ML. *Attachment and barriers to cervical screening*. *J Health Psychol* 2012;0:1-10.
- [26] Cooper ML, Shaver PR, Collins NL. *Attachment styles, emotion regulation, and adjustment in adolescence*. *J Pers Soc Psychol* 1998;74:1380-97.
- [27] Gentzler AL, Kerns KA. *Associations between insecure attachment and sexual experiences*. *Pers Relat* 2004;11:249-65.
- [28] Bogaert AF, Sadava S. *Adult attachment and sexual behavior*. *Pers Relat* 2002;9:191-204.
- [29] Clifford GM, Smith JS, Munoz N, et al. *Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis*. *Br J Cancer* 2003;88:63-73.
- [30] Xu J, Kochanek MA, Murphy SL, et al. *Deaths: final data for 2007*. *National Vital Statistics Reports* 2010:58.
- [31] Cates JR, Herndon NL, Schulz SL, et al. *Our voices, our lives, our futures: youth and sexually transmitted diseases*. Chapel Hill, NC: School of Journalism and Mass Communication. University of North Carolina: Chapel Hill 2004.
- [32] Ciechanowski PS. *As fundamental as nouns and verbs? Towards an integration of attachment theory in medical training*. *Med Educ* 2010;44:122-4.
- [33] Larose S, Bernier A, Soucy N, et al. *Attachment style dimensions, network orientation, and the process of seeking help from college teachers*. *J Soc Pers Relat* 1999;16:225-47.
- [34] Schmidt S, Blank T, Bellizzi K, et al. *The relationship of coping strategies, social support, and attachment style with posttraumatic growth in cancer survivors*. *J Health* 2012;17:1033-40.
- [35] Mikulincer M, Florian V. *The relationship between adult attachment styles and emotional and cognitive reactions to stressful events*. In: Simpson JA, Rholes WS, eds. *Attachment Theory and Close Relationships*. New York: Guilford Press 1998; pp. 143-65.
- [36] Mikulincer M, Florian V. *Appraisal of and coping with a real-life stressful situation: the contribution of attachment styles*. *Pers Soc Psychol Bull* 1995;21:406-14.
- [37] Yi, JK. *Factors associated with cervical cancer screening behavior among Vietnamese women*. *J Community Health* 1994;19:189-200.
- [38] Borrás JM, Guillen M, Sanchez V, et al. *Educational level, voluntary private health insurance and opportunistic cancer screening among women in Catalonia (Spain)*. *Eur J Cancer Prev* 1999;8:427-34.
- [39] Ho IK, Dinh KT. *Cervical cancer screening among Southeast Asian American women*. *J Immigr Minor Health* 2011;13:49-60.
- [40] Cesar J, Horta B, Gomes G, et al. *Factors associated with non-participation in screening for cervical cancer in Southern Brazil*. *Cad Saúde Pública* 2002;19:1365-72.

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ORIGINAL ARTICLE

Antimicrobial potential of Sicilian honeys against commensal *Escherichia coli* and pathogenic *Salmonella* serovar Infantis

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Key words

Honey • *Escherichia coli* • *Salmonella*

Summary

Introduction. The purpose of this study was to investigate the antibacterial effect of 71 locally produced honeys from different botanical sources collected from apiarist's open markets in Sicily.

Methods. Antimicrobial activity was determined against *Escherichia coli* (ATCC 25922) and *Salmonella* serovar *Infantis* (ATCC 1523) by an agar-diffusion assay from the estimation of the diameter of the inhibition zone produced by the honeys. Statistically significant differences ($P < .000$) regarding inhibition were observed for the honeys tested.

Results. The chestnut and polyfloral honey samples exhibited the largest and highest inhibition (diameter of the inhibition

zone > 25 mm) against both *E. coli* and *S. Infantis*. The honey of oregano origin showed intermediate or low activity against *E. coli* and *S. Infantis*, respectively. Prickly pear and erica honeys showed no antimicrobial activity against the two reference strains.

Discussion. The results may partially suggest the usefulness of the Sicilian honeys on treating multi-resistant enterobacteria. In light of the enormous potential for application of honey in the clinical practice, it is important that research continues not only into those honeys well recognized as antimicrobial, but also into other locally produced and yet untested honeys.

Introduction

A re-evaluation of the therapeutic use of ancient remedies such as plants and plant-based products could be important in preventing bacterial antibiotic resistance. In fact, antibiotics are crucial in reducing the global burden of infectious diseases. Anyway, their overuse and misuse in human clinical practice as well as in veterinary medicine has been the direct cause of the emergency of multiple antibiotic resistant bacteria worldwide. One non-conventional medical treatment that has recently received much interest is honey. Honey is produced by honeybees of the genera *Apis mellifera* and *Meliponinae* [1] from many sources, and its antimicrobial activity varies greatly with processing and origin, depending on the natural vegetative flowers blooming in different seasons and in different places [1-3].

Commensal enteric bacteria in particular constitute reservoirs of genes determining antibiotic resistance [4, 5]. Especially when antimicrobial exposure occurs, commensal *Escherichia coli* can transfer their antibiotic resistance in the gastrointestinal environment to the other enterobacteria, including various *Salmonella* serovars, which are the most common bacterial pathogens causing foodborne diseases in developing as well as in developed countries [4-7]. Previous studies conducted at local scale in Sicily (South Italy) have described high prevalence of multiresistant *E. coli* together with the emergence of ampicillin resistance in *E. coli* and *Salmonella* spp. iso-

lates from wastewater and clinical specimens [8, 9]. Thus, from a public health perspective, alternative therapies, such as honey, should draw the public's interest. In Sicily, in particular, the geomorphology and the diversity of flora give the opportunity to the local black honeybees (*Apis mellifera* ssp. *sicula*) of producing a wide variety of honeys from blossoming trees, shrubs and flowers which gives to the final product special sensorial properties. Anyway, despite the variety and recognized quality, as far as we know, only episodic interest has been accorded to this subject in Italy [10]. Therefore, in order to verify whether in future the clinical use of Sicilian honeys could be useful in controlling the spread of antibiotic resistances in enterobacteria, in the current study we sought to study under experimental conditions the antibacterial potency of unpasteurized honeys against *E. coli* and *Salmonella*.

Methods

AREA OF STUDY AND HONEY SAMPLES

The area of study belongs to Sicily, located in the South of Italy, where the autochthon vegetation consists of various spontaneous shrubs and cultivated plants.

Seventy-one honey samples were collected during the 2011 flowering season from apiarist's open markets in two different geographical districts, being 18 samples from centre zone and 53 samples from South-East zone. The honey samples were originated from different bo-

tanical sources, following: 12 polyfloral, 13 of chestnut (*Castanea sativa*), 11 of orange (*Citrus aurantium*), 9 of eucalyptus (*Eucalyptus*), 9 of thyme (*Thymus vulgaris*), 6 of Spanish esparcet (*Hedysarum coronarium*), 4 of citrus (*Citrus limonum*), 4 of carob (*Ceratonia siliqua*), 1 of erica (*Erica vulgaris*), 1 of oregano (*Origanum vulgare*) and finally 1 of prickly pear (*Opuntia vulgaris*) origin.

EVALUATION OF ANTIBACTERIAL ACTIVITY OF HONEYS AND BACTERIAL STRAINS TESTED

An agar diffusion method was used as described above to assess the antibacterial activity of the selected honeys against two reference strains: *Escherichia coli* ATCC 25922 and *Salmonella* serovar Infantis ATCC 1523, which are both susceptible to a wide range of antimicrobials, grow well at low temperatures, and have been shown to be stable in the laboratory following multiple passes on artificial media.

PREPARATION OF THE ASSAY PLATES

E. coli and *S. Infantis* strains were inoculated into 10 mL of tryptic soy broth (TSB; Biolife, Milano, Italy) and incubated at 37°C for 18 h until growth was 0.5 optical density (450 nm). Cultures of 100 µL were added to 18 mL of Mueller-Hinton Agar (Oxoid LTD; Basingstoke, Hampshire, England) previously cooled in a 50°C water bath for 30 min and immediately poured onto Petri plates, one bacterial culture per plate. A grid containing four 25 x 25 mm squares was drawn on the underside of the plates for the deposition of the honey samples as mentioned above. The plates were placed upside-down at 4°C for 24 h before being used the day after.

HONEY SOLUTIONS

All the honey samples were stored at room temperature. Primary honey solutions were prepared by adding 10 g of each well mixed honey to 10 mL of sterile distilled water and placed at 37°C for 30 minutes to aid mixing. To prepare secondary honey solutions, 1 mL of each primary solution was added to 1 mL of sterile distilled water. Aliquots of 100 µL of each honey secondary solution were deposited at the centre of the squares drawn on the assay plates, one aliquot per square of the different assay plates. For each honey secondary solution a control plate that contained no strain culture was prepared. Plates were incubated at 37°C for 24 h.

Antimicrobial activity was determined from the estimation of the diameter of the inhibition zone produced by the honey samples, following: highest activity, diameter > 25 mm; intermediate activity, diameter ≥ 12 mm and ≤ 25 mm; lowest activity, diameter < 12 mm.

For each honey sample the experiments were repeated twice.

STATISTICAL ANALYSIS

The differences between the antibacterial activity against *E. coli* and *S. Infantis* for each honey in results were analyzed by the Chi square test in the statistical package R (<http://www.r-project.org>). A critical value of $p < .000$ was considered statistically significant.

Results

The results of the assays of antibacterial activity of the 71 honeys used in this study are shown in Table I. Statistically significant differences regarding inhibition were observed for the honeys tested with the chestnut and polyfloral honey samples exhibiting the largest and highest inhibition to the two reference strains. The honey of oregano origin showed intermediate or low activity against *E. coli* and *S. Infantis*, respectively. Finally, prickly pear and erica honeys showed no antimicrobial activity against the two reference strains tested (*E. coli*: Chi-square 65.96, df 10, $p = .000$; *S. Infantis*: Chi-square 74.53, df 10, $p = .000$).

Discussion

Honey has been increasingly drawing the public's interest as alternative therapeutic remedy against a wide range of bacteria including some antibacterial-resistant species [11, 12]. In particular, in controlled susceptibility tests, most gastrointestinal bacteria are susceptible to

Tab. I. Antibacterial activity of the 71 honey samples tested against *Escherichia coli* and *Salmonella* serovar Infantis.

Type of honey	Antibacterial activity			
	N	<i>E. coli</i>	N	<i>S. Infantis</i>
Chestnut	10	H ¹	10	H
(N.13)	3	L ³	1	I ²
			2	L
Polyfloral	9	H	11	H
(N.12)	3	R ⁴	1	I
Orange	7	H	11	H
(N.11)	1	I		
	3	L		
Eucalyptus	6	H	7	H
(N.9)	1	L	1	L
	2	R	1	R
Thyme	6	H	6	H
(N.9)	3	R	1	L
Spanish esparcet	4	H	3	H
(N.6)	2	R	1	L
			2	R
Citrus	3	H	2	H
(N.4)	1	R	2	R
Carob	1	H	2	H
(N.4)	3	R	2	R
Erica	1	R	1	R
(N.1)				
Oregano	1	I	1	L
(N.1)				
Prickly pear	1	R	1	R
(N.1)				

¹ = highest activity, diameter > 25 mm

² = intermediate activity, diameter ≥ 12 mm and ≤ 25 mm

³ = lowest activity, diameter < 12 mm

⁴ = no activity

the antimicrobial activity of manuka honey but not to artificial honey [13].

Nonetheless, it has also been suggested that other honeys, both commercially and locally produced, have equivalent activity for some, but not all, bacteria [14-16]. In the present study we have described that Sicilian locally obtained unprocessed honeys may be active against *E. coli* and *S. Infantis*. In fact, our data show that all but two (erica and prickly pear) of the 71 honey samples tested have some antibacterial action, the activity ranging from 'high' to 'low'. These results are in accordance with a previous study on the concentrations of the major 1,2-dicarbonyl compounds in Sicilian commercial honey samples from 12 different floral origins [17].

Obviously, careful should be applied in generalizing these results to all the Sicilian honeys for some main reasons. First, the relatively low number of honey samples tested. Anyway, the overall good activity showed by the majority of the honeys (chestnut, polyfloral, orange, eucalyptus, thyme and Spanish esparcet), which together account to 84.5% of the honey samples tested, could indicate a good activity of Sicilian honeys against enterobacteria.

Second, although significant inhibition of bacterial growth was noted for the majority of the honeys tested, it is doubtful whether the activity observed under experimental conditions would be clinically significant. In fact, in this study we have used an agar-diffusion assay rather than an agar dilution method that could better mimic the situation where the honey dress directly in contact with the infected mucosa [18]. For this reason, we think that further research is required to assess the correlation between the described antibacterial activity *in vitro* and the actions *in vivo* of the Sicilian honeys.

Third, our results underline that *E. coli* and *S. Infantis* showed also a certain degree of resistance to some of the honeys tested. Some bacterial species can be inhibited by low levels of osmolarity, so inhibition by honey may be due to the sugar content rather than to hydrogen peroxide or non-peroxide factors [19]. From this perspective, further experimental essays could be useful in order to stand-

ardize the reported antibacterial activities of the Sicilian honeys. In particular, the use of a reference antiseptic or the comparison with an artificial honey as a reference could be useful in distinguishing the efficacy of antibacterial factors other than the osmolarity. Anyway, there is evidence that the activity of honeys can vary greatly among different floral types [20]. Moreover, even the antibacterial activity of honeys sharing the same floral origin could greatly differ in activity depending, for example, from the storing conditions. Thus, because the antibacterial activity of honey is sensitive to light and to heat [21], differences in the observed antibacterial activity could be due to the fact that our honey samples were stored at room temperature and not in a dark refrigerator.

Finally, although the limited number of strains considered in our study their sensitivity to Sicilian honeys may partially suggest the usefulness of these honeys on treating multi-resistant *E. coli* and *Salmonella* spp. previously isolated from wastewater and clinical specimens in Sicily [8, 9]. For this reason, we think that it would be of value to further investigate the potency of these honeys with more antibiotic resistant bacterial species in future in the view of a possible clinical use.

Conclusions

In conclusion, in light of the enormous potential for application of honey in the clinical practice, it is important that research continues not only into those honeys well recognized as antimicrobial, but also into other locally produced and yet untested honeys. Although the number of antibacterial resistant strains that have been tested with honey in our study is limited and although it was not been evaluated whether or not bacteria would eventually develop resistance to honey, the sensitivity of *E. coli* and *S. Infantis* to the honeys tested may partially suggest the usefulness of the Sicilian honeys on treating multi-resistant enterobacteria. It would be of value to further investigate the potency of the Sicilian honeys with more antibiotic resistant bacterial species in future.

References

- [1] Manyi-Loh CE, Clarke AM, Ndip RN. *An overview of honey: therapeutic properties and contribution in nutrition and human health*. Afr J Microbiol Res 2011;5:844-52.
- [2] Mulu A, Tessema B, Derby F. *In vitro assessment of the antimicrobial potential of honey on common human pathogens*. Ethiop J Health Dev 2004;18:107-11.
- [3] Tan HT, Rahman RA, Gan SH, et al. *The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey*. BMC Compl Alternative Med 2009;9:34.
- [4] Shehabi AA, Odeh JF, Fayyad M. *Characterization of antimicrobial resistance and class 1 integrons found in Escherichia coli isolates from human stools and drinking water sources in Jordan*. J Chemother 2006;18:468-72.
- [5] Su J, Shi L, Yang L, et al. *Analysis of integrons in clinical isolates of Escherichia coli in China during the last six years*. FEMS Microbiol Lett 2006;254:75-80.
- [6] Smith JL, Drum DJV, Dai Y, et al. *Impact of antimicrobial usage on antimicrobial resistance in commensal Escherichia coli strains colonizing broiler chickens*. Appl Environ Microbiol 2007;73:1404-14.
- [7] Hawkey PM, Jones AM. *The changing epidemiology of resistance*. J Antimicrob Chemother 2009;64:3-10.
- [8] Pignato S, Coniglio MA, Faro G, et al. *Plasmid-mediated multiple antibiotic resistance of Escherichia coli in crude and treated wastewater used in agriculture*. J Water Health 2009;7:251-8.
- [9] Pignato S, Coniglio MA, Faro G, et al. *Molecular epidemiology of ampicillin resistance in Salmonella spp. and Escherichia coli from wastewater and clinical specimens*. Foodborne Pathog Dis 2010;7: 945-51.
- [10] Tenore GC, Ritieni A, Campiglia P, et al. *Nutraceutical potential of monofloral honeys produced by the Sicilian black honeybees (Apis mellifera ssp. sicula)*. Food Chem Toxicol 2012;50:1955-61.
- [11] Badawy OFH, Shafii SSA, Tharwatt EE, et al. *Antibacterial activity of bee honey and its therapeutic usefulness against Escherichia coli O157:H7 and Salmonella Typhimurium infection*. Rev Sci Tech 2004;23:1011-22.

- [12] Adebolu TT. *Effect of natural honey on local isolates of diarrhea-causing bacteria in southwestern Nigeria*. Afri J Biotech 2005;4:1172-4.
- [13] Lin SM, Molan PC, Cursons RT. *The controlled in vitro susceptibility of gastrointestinal pathogens to the antibacterial effect of manuka honey*. Eur J Clin Microbiol Infect Dis 2011;30:569-74.
- [14] Nzeako BC, Hamdi J. *Antimicrobial potential of honey on some microbial isolates*. Res J Med Sci 2000;2:75-9.
- [15] Ceyhan N, Ugur A. *Investigation of in vitro antimicrobial activity of honey*. Riv Biol 2001;94:363-71.
- [16] Ahmed M, Djebli N, Aissat S, et al. *Antiradical activity and total phenolics of Algerian honeys and antibacterial effect against Gram-negative bacteria*. J Microb Biochem Technol 2012;4:152-6.
- [17] Arena E, Ballistreri G, Tomaselli F, et al. *Survey of 1,2-dicarbonyl compounds in commercial honey of different floral origin*. J Food Sci 2011;76:1203-10.
- [18] Lusby PA, Coombes AL, Wilkinson JM. *Bactericidal activity of different honeys against pathogenic bacteria*. Arch Med Res 2005;36:464-7.
- [19] Windsor S, Pappalardo M, Brooks P, et al. *A convenient new analysis of dihydroxyacetone and methylglyoxal applied to Australian Leptospermum honeys*. J Pharmacogn Phytother 2012;4:6-11.
- [20] Alnaimat S, Wainwright M, Al'Abri K. *Antibacterial potential of honey from different origins: a comparison with manuka honey*. J Microbiol Biotechnol Food Sci 2012;1:1328-38.
- [21] Al-Waili NS. *Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva*. J Med Food 2004;7:210-22.

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Volume 54, 2013 - General index

Issue 1 – March 2013

Review

- Influenza vaccination in HIV-positive subjects: latest evidence and future perspective
A. Ceravolo, A. Orsi, V. Parodi, R. Rosselli, F. Ansaldi 1

Short review

- Health promotion or pharmacological treatment for chronic diseases?
M.F. Allam, M.A. Ortiz Arjona 11

Original articles

- An opportunistic pre-diabetes screening program offered with existing hypertension screening
M.T. Iqbal 14
- Piercing and tattooing in high school students of Veneto region: prevalence and perception of infectious related risk
S. Majori, F. Capretta, T. Baldovin, M. Busana, V. Baldo & Collaborative Group 17
- Pseudomonas aeruginosa* infection among cystic fibrosis and ICU patients in the referral Children Medical Hospital in Tehran, Iran
Z. Movahedi, B. Pourakbari, S. Mahmoudi, F. Sabouni, M.T. Haghi Ashtiani, R. Hosseinpour Sadeghi, S. Mamishi 24
- Impact of immigration on burden of Tuberculosis in Umbria: a low-incidence Italian region with high immigrants rates
N. Buonora, M. Chiavarini, L. Salmasi, M.D. Giaimo, L. Minelli 29
- Determinants of utilization of traditional bone setters in Ilorin, North Central Nigeria
S.A. Aderibigbe, S.R. Agaja, J.O. Bamidele 35
- Correlation between physical activity and sedentary behavior with healthy and unhealthy behaviors in Italy and Tuscan region: a cross sectional study
G. Lazzeri, E. Azzolini, A. Pammolli, D.R. De Wet, M.V. Giacchi 41
- Asymptomatic malaria parasitaemia using rapid diagnostic test in unbooked pregnant women in rural Ondo-south district, Nigeria
D.U. Nwaneri, O.A. Adeleye, A.B. Ande 49
- Baking soda and salt in bakeries of Mehrdasht (Najafabad), Isfahan, Iran: a survey on a typical rural population in a developing country
M. Rezaiimofrad, F. Rangraz Jeddi, Z. Azarbad 53
- Case report**
- Dermatosis caused by *Corythuca ciliata* (Say, 1932) (Heteroptera, Tingidae). Diagnostic and clinical aspects of an unrecognized pseudoparasitosis
M. Dutto, M. Bertero 57

Issue 2 – June 2013

Reviews

- HPV related diseases in males: a heavy vaccine-preventable burden
P. Canepa, A. Orsi, M. Martini, G. Icardi 61

- Preventive capacity of allergen immunotherapy on the natural history of allergy
C. Incorvaia 71

Original articles

- Improving environmental quality in an operating room: clinical outcomes and economic implications
M. Sartini, A.M. Spagnolo, D. Panatto, F. Perdelli, M.L. Cristina 75
- Clinical risk factors and bronchoscopic features of invasive aspergillosis in Intensive Care Unit patients
M. Aliyali, M.T. Hedayati, M.R. Habibi, S. Khodavaisy 80
- Rs12979860 and rs8099917 single nucleotide polymorphisms of interleukin-28B gene: simultaneous genotyping in Caucasian patients infected with hepatitis C virus
L. Sticchi, A. Di Biagio, E. Rappazzo, M. Setti, G. De Rosa, L. De Hoffer, L. Nicolini, R. Prinapori, B. Bruzzzone 83
- Clonal spread of vancomycin resistance *Enterococcus faecalis* in an Iranian referral pediatrics center
B. Pourakbari, S. Mahmoudi, M. Kamali Aghdam, F. Sabouni, H. Eshaghi, S. Alizadeh, S. Mamishi 87
- Analysis of errors in histology by root cause analysis: a pilot study
P. Morelli, E. Porazzi, M. Ruspini, U. Restelli, G. Banfi 90
- Tobacco smoking among students in an urban area in Northern Italy
D. Panatto, D. Amicizia, A. Domnich, P.L. Lai, M.L. Cristina, A. Signori, S. Boccalini, K. Sulaj, R. Gasparini 97
- To start and quit smoking cigarettes: an evaluation of students in a Nigerian city
O.A. Atoyebe, D.O. Ibirongbe, O.A. Babatunde, O.E. Atoyebe 104
- Impact of anti-tobacco warning labels on behaviour of tobacco users in one of the cities of Gujarat, India
V.R. Shah, V.R. Dave, K.N. Sonaliya 109

Risk perception of sexually transmitted diseases and teenage sexual behaviour: attitudes towards in a sample of Italian adolescents

M. Bergamini, A. Cucchi, E. Guidi, A. Stefanati, B. Bonato, S. Lupi, P. Gregorio 114

Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011

M. Vahedi, M. Nasrolahei, M. Sharif, A.M. Mirabi 120

The nurses' point of view

Paediatric nurses' perception of the child-family dyad's autonomy in managing a chronic disease situation: the experience of an Italian Paediatric Department

A. Bagnasco, P. Petralia, S. Furnari, S. Ghio, S. Calza, L. Sasso 124

Issue 3 – September 2013

Reviews

Operating theatre quality and prevention of surgical site infections

A.M. Spagnolo, G. Ottria, D. Amicizia, F. Perdelli, M.L. Cristina 131

Biphosphonates-associated osteonecrosis of the jaw: the role of gene-environment interaction

A. Izzotti, M. Menini, A. Pulliero, G. Dini, C. Cartiglia, P. Pera, D. Baldi 138

Original articles

Health workers sensitization: effects on perceived quality of immunization services among mothers of under five children in Ilorin, North Central Nigeria

O.O. Goodman, S.A. Aderibigbe, O.O. Sekoni, G.K. Osagbemi, T.M. Akande 146

Personal hygiene among primary school children living in a slum of Kolkata, India

M. Sarkar 153

Frequency and profile of induced abortions: hospital based study in tertiary hospitals in Egypt

H. Harb, I. Habil 159

Testicular-self examination among Nigerian adolescent secondary school boys: knowledge, attitudes and practices

A.N. Onyiriuka, F.E. Imoeb 163

Sequence based typing of *Legionella pneumophila* sg 1 isolated in nosocomial acquired infections in Apulia, Southern Italy

R. Iatta, T. Cuna, O. De Giglio, M.T. Montagna, C. Napoli 167

Urinary tract infection among intellectual disability individuals "Etiology and antibiotic resistance patterns" in rehabilitation centers of Mazandaran province, Northern Iran

M. Nasrolahei, M. Poorhagibagher, M. Vahedi, I. Maleki 170

Anti-rods and rings autoantibodies can occur in the hepatitis c-naïve population

Y. Shaikh, A. Krantz, Y. El-Farra 175

Suspension of mandatory vaccination and public health preserving: Rovigo Local Health Unit experience after appliance of Veneto regional law 7/2007

M. Cattarin, M. Bellè, M. Bergamini, L. Gallo 181

Attitude and knowledge of Iranian female nurses about Human Papillomavirus infection and cervical cancer: a cross sectional survey

S. Mojahed, M. Karimi Zarchi, M. Bokaie, T. Salimi 187

Issue 4 – December 2013

Original articles

Adolescents: which risks for their life and health?

E. Balocchi, G. Chiamenti, A. Lamborghini 191

Stress among medical Saudi students at College of Medicine, King Faisal University

A.G. Abdel Rahman, B.N. Al Hashim, N.K. Al Hiji, Z. Al-Abbad 195

Prevalence survey of healthcare-associated infections and antimicrobial use at the University Hospital "Paolo Giaccone", Palermo, Italy

I. Sinatra, L. Carubia, V. Marchese, L. Aprea, N. D'Alessandro, C. Mammì, M.V. Torregrossa 200

Staphylococcus aureus infections in children in an Iranian referral pediatric Hospital

F. Sabouni, R. Ranjbari, B. Pourakbari, S. Mahmoudi, M. Teymuri, M. Taghi Haghi Ashtiani, Z. Movahedi, S. Mamishi 205

Social and interpersonal relationship modifications after renal transplant. A statistic and epidemiologic evaluation

F. Famà, E. Micali, C. Linard, M.D. Venuti, G. Costantino, M. Giofrè-Florino 208

A study on occupational exposure of Sicilian farmers to *Giardia* and *Cryptosporidium*

F. Di Piazza, M.A. Di Benedetto, C.M. Maida, S. Glorioso, G. Adamo, T. Mazzola, A. Firenze 212

Psychosocial predictors of barriers to cervical cancer
screening among Iranian women: the role of attachment
style and social demographic factors
K. Hajjalizadeh, H. Ahadi, F. Jomehri, M. Rahgozar 218

Antimicrobial potential of Sicilian honeys against
commensal *Escherichia coli* and pathogenic *Salmonella*
serovar Infantis
M.A. Coniglio, G. Faro, G. Giammanco, S. Pignato,
M. Marranzano 223

Volume 54, 2013 - Author index

- Abdel Rahman A.G., 195
 Adamo G., 212
 Adeleye O.A., 49
 Aderibigbe S.A., 35, 146
 Agaja S.R., 35
 Ahadi H., 218
 Akande T.M., 146
 Al Hashim B.N., 195
 Al Hiji N.K., 195
 Al-Abbad Z., 195
 Aliyali M., 80
 Alizadeh S., 87
 Allam M.F., 11
 Amicizia D., 97, 131
 Ande A.B., 49
 Ansaldi F., 1
 Aprea L., 200
 Atoyebi O.A., 104
 Atoyebi O.E., 104
 Azarbad Z., 53
 Azzolini E., 41

 Babatunde O.A., 104
 Bagnasco A., 124
 Baldi D., 138
 Baldo V., 17
 Baldovin T., 17
 Balocchi M., 191
 Bamidele J.O., 35
 Banfi G., 90
 Bellè M., 181
 Bergamini M., 114
 Bergamini M., 181
 Bertero M., 57
 Boccalini S., 97
 Bokaie M., 187
 Bonato B., 114
 Bruzzzone B., 83
 Buonora N., 29
 Busana M., 17

 Calza S., 124
 Canepa P., 61
 Capretta F., 17
 Cartiglia C., 138
 Carubia L., 200
 Cattarin M., 181
 Ceravolo A., 1
 Chiamenti G., 191
 Chiavarini M., 29
 Coniglio M.A., 223
 Costantino G., 208
 Cristina M.L., 75, 97, 131
 Cucchi A., 114
 Cuna T., 167

 D'Alessandro N., 200
 Dave V.R., 109

 De Giglio O., 167
 De Hoffer L., 83
 De Rosa G., 83
 De Wet D.R., 41
 Di Benedetto M.A., 212
 Di Biagio A., 83
 Di Piazza F., 212
 Dini G., 138
 Domnich A., 97
 Dutto M., 57

 El-Farra Y., 175
 Eshaghi H., 87

 Famà F., 208
 Faro G., 223
 Firenze A., 212
 Furnari S., 124

 Gallo L., 181
 Gasparini R., 97
 Ghio S., 124
 Giacchi M.V., 41
 Giaimo M.D., 29
 Giammanco G., 223
 Giofrè-Florio M., 208
 Glorioso S., 212
 Goodman O.O., 146
 Gregorio P., 114
 Guidi E., 114

 Habibi M.R., 80
 Habil I., 159
 Haghi Ashtiani M.T., 24
 Hajializadeh K., 218
 Harb H., 159
 Hedayati M.T., 80
 Hosseinpour Sadeghi R., 24

 Iatta R., 167
 Ibirongbe D.O., 104
 Icardi G., 61
 Imoebé F.E., 163
 Incorvaia C., 71
 Iqbal M.T., 14
 Izzotti A., 138

 Jomehri F., 218

 Kamali Aghdam M., 87
 Karimi Zarchi M., 187
 Khodavaissy S., 80
 Krantz A., 175

 Lai P.L., 97
 Lamborghini A., 191
 Lazzeri G., 41
 Linard C., 208

 Lupi S., 114

 Mahmoudi S., 24, 87, 205
 Maida C.M., 212
 Majori S., 17
 Maleki I., 170
 Mamishi S., 24, 87, 205
 Mammia C., 200
 Marchese V., 200
 Marranzano M., 223
 Martini M., 61
 Mazzola T., 212
 Menini M., 138
 Micali E., 208
 Minelli L., 29
 Mirabi A.M., 120
 Mojahed S., 187
 Montagna M.T., 167
 Morelli P., 90
 Movahedi Z., 24, 205

 Napoli C., 167
 Nasrolahei M., 120, 170
 Nicolini L., 83
 Nwaneri D.U., 49

 Onyiriuka A.N., 163
 Orsi A., 1, 61
 Ortiz Arjona M.A., 11
 Osagbemi G.K., 146
 Ottria G., 131

 Pammolli A., 41
 Panatto D., 75, 97
 Parodi V., 1
 Pera P., 138
 Perdelli F., 75, 131
 Petralia P., 124
 Pignato S., 223
 Poorhagibagher M., 170
 Porazzi E., 90
 Pourakbari B., 24, 87, 205
 Prinapori R., 83
 Pulliero A., 138

 Rahgozar M., 218
 Rangraz Jeddi F., 53
 Ranjbari R., 205
 Rappazzo E., 83
 Restelli U., 90
 Rezaei-mofrad M., 53
 Rosselli R., 1
 Ruspini M., 90

 Sabouni F., 24, 87, 205
 Salimi T., 187
 Salmasi L., 29
 Sarkar M., 153

Sartini M., 75	Signori A., 97	Taghi Haghi Ashtiani M., 205
Sasso L., 124	Sinatra I., 200	Teymuri M., 205
Sekoni O.O., 146	Sonaliya K.N., 109	Torregrossa M.V., 200
Setti M., 83	Spagnolo A.M., 75, 131	
Shah V.R., 109	Stefanati A., 114	Vahedi M., 120, 170
Shaikh Y., 175	Sticchi L., 83	Venuti M.D., 208
Sharif M., 120	Sulaj K., 97	

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BOOKS:

Smith DW. *Recognizable patterns of human malformation*. Third Edition. Philadelphia: WB Saunders Co. 1982.

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CONTENTS

ORIGINAL ARTICLES

Adolescents: which risks for their life and health? E. Balocchi, G. Chiamenti, A. Lamborghini	191
Stress among medical Saudi students at College of Medicine, King Faisal University A.G. Abdel Rahman, B.N. Al Hashim, N.K. Al Hiji, Z. Al-Abbad	195
Prevalence survey of healthcare-associated infections and antimicrobial use at the University Hospital “Paolo Giaccone”, Palermo, Italy I. Sinatra, L. Carubia, V. Marchese, L. Aprea, N. D'Alessandro, C. Mammina, M.V. Torregrossa	200
<i>Staphylococcus aureus</i> infections in children in an Iranian referral pediatric Hospital F. Sabouni, R. Ranjbari, B. Pourakbari, S. Mahmoudi, M. Teymuri, M. Taghi Haghi Ashtiani, Z. Movahedi, S. Mamishi	205
Social and interpersonal relationship modifications after renal transplant. A statistic and epidemiologic evaluation F. Famà, E. Micali, C. Linard, M. Dorian Venuti, G. Costantino, M. Gioffrè-Florio	208
A study on occupational exposure of Sicilian farmers to <i>Giardia</i> and <i>Cryptosporidium</i> F. Di Piazza, M.A. Di Benedetto, C.M. Maida, S. Glorioso, G. Adamo, T. Mazzola, A. Firenze	212
Psychosocial predictors of barriers to cervical cancer screening among Iranian women: the role of attachment style and social demographic factors K. Hajializadeh, H. Ahadi, F. Jomehri, M. Rahgozar	218
Antimicrobial potential of Sicilian honeys against commensal <i>Escherichia coli</i> and pathogenic <i>Salmonella</i> serovar <i>Infantis</i> M.A. Coniglio, G. Faro, G. Giammanco, S. Pignato, M. Marranzano	223



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