ORIGINAL ARTICLE

Extended-spectrum ß-lactamases Klebsiella pneumoniae: multimodal infection control program in Intensive Care Units

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Key words

Drug resistance multiple bacterial • Infection control • Intensive care units

Summary

Introduction. *The aims of our study were to investigate a nosocomial spread of an extended-spectrum β-lactamases -Klebsiella pneumoniae cluster at a University teaching hospital in Italy, to describe, and to monitor the implementation of a multimodal infection control program in two mixed ICUs.*
Methods. *During the 1 October 2005-30 September 2006 period, 79 colonized patients have been identified. Isolates were genotyped by pulsed-field gel electrophoresis (PFGE). A mutimodal infection control program with monitoring of alcohol-based hand rub was performed in Intensive Care Units (ICU A and ICU B).*
Results. *The epidemiological investigation and PFGE showed a horizontal transmission of the same PFGE genotype, with the isolation of the outbreak strain on the hand of one healthcare operator.*
Alcohol based hand rub was adopted in ICU A on 18 March 2006, in addition to hand washing with plain or antiseptic soap. ICU B did not change its hand hygiene habits. Following the implementation of the program, the incidence density rate (IDR) in ICU A fell down from 4.50 to 1.68/1000 patient days.
Discussion and conclusions. *Our findings confirm the important role of personnel in cross-transmission.*
Moreover the inbuilt control group involuntarily offered by the delaying of the intervention in ICU B has given the opportunity to verify the epidemiological association between the actual implementation of infection control practices and the outbreak control.

Introduction

In recent years, multidrug-resistant *Klebsiella pneumoniae* producing extended-spectrum β-lactamases (ESBL) have been increasingly recognized as a cause of nosocomial outbreaks worldwide, also because of the facility to acquire and to transfer the plasmidic resistances [1, 2]. The Intensive Care Unit (ICU) environment often makes this part of the hospital a focus for the emergence and spread of many antimicrobial-resistant pathogens, because patients are commonly exposed to broad-spectrum antimicrobial agents and there are ample opportunities for exogenous bacterial colonization due to high rate of interventions and wide use of invasive medical devices [1, 3, 4]. In fact, even though some epidemiological investigations revealed a common source for the outbreaks [1], other evidences suggest that transient carriage on the hands of health care workers can represent a more important way of transfer from patient to patient [3, 5]. Therefore, the benefits of hand hygiene in reducing the risk of cross contamination in hospitals are well established [6]; however, infection control professional, knows that the simple educational intervention may be poor in predicting hand hygiene performance [7]. To overcome these difficulties, data on the utilization of hand hygiene products may serve as a simple indicator of hand hygiene performance, and thereby as a tool for hospital infection control [6]. Moreover, the adoption of infection control precaution has been reported as an effective way to control an outbreak [8, 9]. The aims of our study were to investigate a nosocomial spread of an ESBL -*Klebsiella pneumoniae* cluster at a University teaching hospital in Italy, to describe, and to monitor the implementation of a multimodal infection control program in two mixed ICU.

Materials and methods

SETTING

The study was carried out in two mixed ICUs of a tertiary care teaching hospital located in Central Italy. The hospital consists of 57 wards, 8 of them in critical area (7 ICUs and 1 Emergency Medicine). The total number of acute-care beds is 893, 152 of them belong to critical area.
Both ICU, A and B, have similar structural features with 12 beds each (one open space, one isolation room and two double rooms) and with the following activity indicators: mean hospital stay of 19.8 vs 17.0 days ($p > 0.05$).

In both ICUs, patients are admitted either with medical and surgical conditions without any kind of selection for the entrance diagnosis. During 2006, 164 patients were hospitalized in ICU A and 181 in ICU B, most of them with surgical DRG (73.8% in ICU A e 77.9% in ICU B).

Surveillance

The outbreak was identified by an active laboratory surveillance system. This computerized system of surveillance, available in the laboratory of clinical microbiology, can identify the sentinel microorganisms on the basis of the epidemiological characteristics of microbial circulation. In case of new resistance profiles, the laboratory alerts the hospital ward, reporting the indication of "multiresistant microorganism", and strongly recommending healthcare workers to take the appropriate infection control measures. Every year a report is made about the microorganisms circulating in "at risk wards" (on the basis of the isolation frequency and severity of the patient clinical conditions) with the relative antimicrobial resistance. During the outbreak, several reports were made every week on the new positive isolations for ESBL -*Klebsiella pneumoniae*. Moreover, since four cases were identified, on January 31st 2006, the Hospital Hygiene Service started an epidemiological outbreak investigation through a revision of the clinical records of the patients with at least one positive isolate of ESBL - *Klebsiella pneumoniae*. Case definition included all patients admitted to the two ICUs, with at least one positive isolate for ESBL -*Klebsiella pneumoniae*. Outbreak investigation started in order to verify the diagnosis; define and identify cases; describe the data in terms of time, place, and person; develop hypotheses; evaluate hypotheses and, eventually, refine them and carry out additional studies; implement control and prevention measures; and communicate findings. Infected patients have been defined according to the Center for Disease Control/ National Healthcare Safety Network (CDC/ NHSN) surveillance definition criteria of health care-associated infection [9]. Patient screening has not been adopted, microbiological testing has been carried out only in suspected cases. Patients information was obtained through chart review; including age, sex, length of stay, admission date, hospital departments of admission, diagnosis of admission, utilization of invasive devices, surgical interventions, microbiological isolates and antibiotic treatment.
Therefore, the surveillance concerned all patients hospitalized from 1 October 2005 to 30 September 2006 in the hospital, in particularly in two ICUs.

Control measures

A team of multidisciplinary professionals from different specialties was constituted by hospital administration, hospital hygiene, infectious diseases, critical care services and microbiology division of laboratory medicine. The team planned and coordinated the type and the timing of multimodal interventions in the two ICUs. Multimodal intervention consisted of epidemiological investigation, environmental and health care workers' hand sampling, added emphasis on adherence to isolation precautions [10] and judicious antimicrobial use has been recommended during the outbreak with multidisciplinary review of local susceptibility patterns (antibiograms), and antimicrobial agents included in the hospital formulary [5], clinical audit, training for critical area healthcare workers about the prevention of nosocomial infections, feedback to healthcare workers about laboratory results, reduction in the number of visitors (from 6 to 2), and reduction of hospitalizations by means of planned neurosurgical interventions (because among ICU's patients this type of surgical intervention is the most frequent). Moreover the use of alcohol-based hand rub has been emphatised in addition to hand washing with plain or antiseptic soap. It is important to underline that in both ICUs the same interventions were performed as either directors and nursing coordinators of both ICUs were part of the multidisciplinary team that planned the interventions. The only different thing was the adoption of the alcohol-based hand rub only in ICU A. In fact, as a proxy indicator of actual adoption of the procedure we have utilized its order from the hospital pharmacy service.
Given the availability of a unique isolation room in each ICU, in case of stay of more cases in the ICUs, functional contact infection control precautions were adopted. In particular, the first control measure has been the definition of designated bed space in shared room, together with cohorting of patients, of healthcare personnel assisting the cases, and of instruments; moreover, continuous disinfection of the patients' bed space, and the adoption of a single trail standing before the patient area including personal protective equipment, to be used by the dedicated healthcare professionals, were adopted.
The environmental sampling included rooms that housed patients colonized with ESBL-*Klebsiella pneumoniae* and were performed on the following surfaces: computer, keyboard and mouse, bed control panel, infusion pump control panel, infusion device control panel, shelving and open trolley's handles, headboard-edge-side of bed, steel shelves, monitor mobile system, clinical record, windowsill, respirator device, water used in container for aspiration tubes. Such samplings, 41 in ICU A and 16 in ICU B, were carried out during the last week of February. Samplings on the hands of 24 health healthcare workers (at work on the day of environmental sampling) of ICU A, and 30 healthcare workers of ICU B were carried out on March 6th, to evaluate the microbial count and presence of *Klebsiella pneumoniae*.

Laboratory analysis

The following cultural media were used: to isolate *Klebsiella pneumoniae*, the MacConKey N3 agar (Oxoid). The identification of *K. pneumoniae* was performed by the automated instrument Vitek II (Bio Mérieux). The isolates susceptibility to amoxillin/ clavulanic acid, cefepime, ceftazidime, cefoxitin, imipenem, meropenem, piperacillin, piperacillin/tazobactam, cotrimoxazole, gentamicin, tobramycin, amikacin and ciprofloxacin

was evaluated using the disc diffusion method on Mueller Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI) criteria [11]. Minimal inhibitory concentrations (MICs) for antibiotics were evaluated using the E-test method and the results expressed in µg/ml were: amoxicillin/clavulanic acid: ≥ 32, cefepime: ≥ 64, ceftazidime: ≥ 64, cefoxitin: ≥ 64, imipenem: 1, meropenem: 1, piperacillin: ≥ 128, piperacillin-tazobactam: ≥ 128, cotrimoxazole: ≥ 320, gentamicin: ≥ 16, tobramycin: ≥ 16, amikacin: ≥ 64 and ciprofloxacin: ≥ 4.

The isolates were send to the Department of Molecular Biology, Section of Microbiology, Siena University, to extract the plasmid DNA, and determine the nature of the CTX-M type enzymes by PCR amplification followed by direct sequencing of the amplicons [12].

Genomic relatedness was investigated by Random Amplification Polymorphic DNA (RAPD) profiling as described previously [13]. Conjugation experiments were performed on solid medium using the E. coli strain MKD-135 (argH rpoB18 rpoB19 recA rpsL) as recipient, as described previously [14]. Transconjugants were selected on Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) containing rifampicin (250 µg/ml) plus cefotaxime (2 µg/ml). Plasmid DNA was extracted using the alkaline lysis method [15]. The nature of the CTX-M-type enzymes was determined by PCR amplification, followed by direct sequencing of the amplicons as described previously [14]. Plasmid profiles of the transconjugants were determined after digestion with the PstI endonuclease (New England Biolabs, Beverly, U.S.A).

Identification and genotyping of multidrug-resistant *Klebsiella pneumoniae* isolates, with similar pattern of antibiotic resistance, underwent to molecular genotypization, performed by pulsed-field gel electrophoresis (PFGE), using conventional procedures and criteria for interpretation [16]. PFGE was performed on 48 strains, corresponding to the first positive isolate of 47 patients and to the strain isolated from the hands of the health care worker. Genomic DNA was prepared in agarose plugs and bacterial DNA was digested with 20 U Xba I restriction enzyme (Invitrogen). PFGE was performed using a Bio-Rad CHEF mapper (Bio-Rad CHEF DR II System). Run time was 24 h at 14°C, using switch times of 10-45 seconds. Molecular size standards were obtained by Sma I digestion of chromosomal DNA of *Staphylococcus aureus* NCTC 8325. The gel was stained with ethidium bromide (10 mg/ml) and photographed under UV light [17, 18].

Statistical analysis

We calculated the Incidence Density Rates per 1000 days of stay (IDR) before and after the adoption of alcohol-based hand rub in ICU A, and ICU B (non participating). Moreover, the correlation between time of stay on ICU and first time of detection of ESBL-*Klebsiella pneumoniae* has been calculated. The monitoring of alcohol based hand rub utilization has been evaluated in terms of L/1000 patient days. Level of significance was set at $p < 0.05$. Analysis was performed by using STATA statistical software (version 8.0).

Results

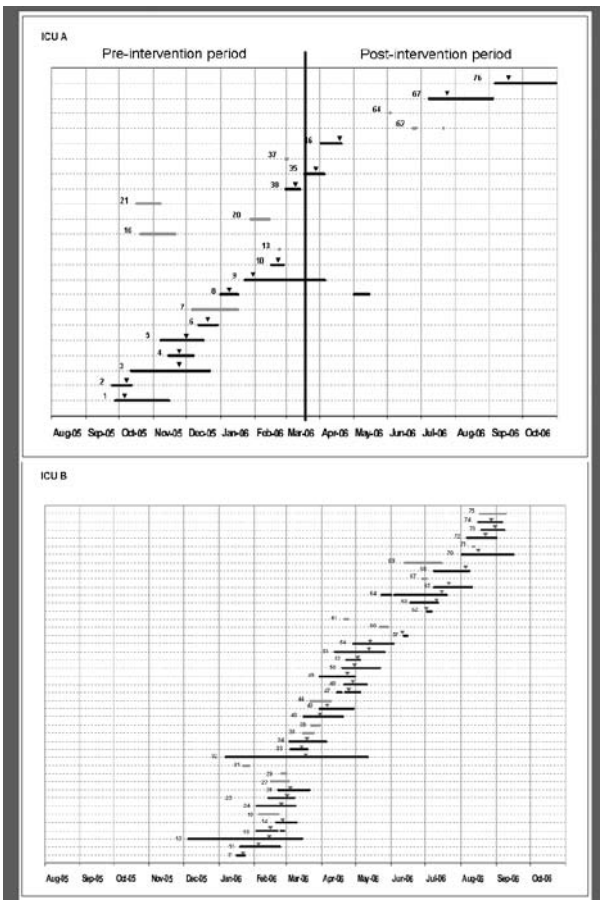
During the study period, 79 hospitalised patients had at least one isolate of ESBL-*Klebsiella pneumoniae*, 60 of them stayed in one of the two ICUs at least for a part of their hospital stay. Altogether, 16 wards were involved (4 in surgical area, 7 in medical area and 5 in critical area), with a maximum increase of 15 cases in March.

Figure 1 shows the period of hospitalization of cases in ICUs A and B.

The first positive isolate of ESBL-*Klebsiella pneumoniae* in ICU B happened after the transfer in this ward of one patient (identified by number "7" in Figure 1) from ICU A.

The demographical and clinical characteristics of patients are listed in Table I.

Fig. 1. Patients' hospital stay in ICU A and in ICU B.



First graphic shows patients' hospital stay in ICU A and in ICU B. Numbers show patient's id. Patients with the first isolate in each ICU are represent by dark line, while patients with the first positive isolate in other wards are represent by light line. Dark indicators mark the first positive isolate date, while light ones show the negative isolate.

Tab. I. Distribution of demographical and clinical characteristics of the patients with MDR Klebsiella pneumoniae (N = 79).

Variables	Total (n)	(%)
Age, years		
Mean (± SD)	57.0 (± 17.8)	
Sex		
Male (%)	51	64.6
Origin		
Community*	62	78.5
Outcome		
Death	16	20.3
Moved to another institute	43	54.4
Admission diagnosis		
cerebral vascular diseases and expansive cerebral processes	35	44.3
polytrauma with cranial trauma	16	20.3
internal diseases	14	17.7
polytrauma without cranial trauma	6	7.6
surgical diseases (except neurosurgery)	4	5.1
septic shock (except MDR Klebsiella pneumoniae)	4	5.1
Devices		
Central venous catheter (CVC)	72	91.1
Urinary catheter (UC)	70	88.6
Nasogastric tube (NGT)	56	70.9
Orotracheal intubation (OTI)	53	67.1
Tracheostomy	37	46.8
Artery catheter	30	38.0
Intracranial Pressure (ICP)	18	22.8
Percutaneous endoscopy gastrostomy (PEG)	15	19.0
External Ventricular drainage (EVD)	14	17.7
Surgical Intervention		
Yes	66	83.5
Neurosurgical intervention	46	69.7
Positive specimen at first isolation**		
Aerial ways	36	40.4
Urine	35	39.3
Blood	11	12.4
Miscellaneous	7	7.9

* patients from their home or from Emergency Department
** 9 patients showed more than one positive material at the first isolation
Aerial ways: bronchus aspirate; bronchoalveolar lavage; bronchial brushing; endotracheal aspirate; sputum; tracheal aspirate.
Urine: Urine culture, culture of urinary catheter tips
Blood: blood culture from Peripheral vein/artery/central venous catheter, culture of central venous catheter
Miscellaneus: vaginal swap, ascitic fluid, skin, gastric aspirate, surgical site swap

The mean time between admission and first positive isolate for ESBL-*Klebsiella pneumoniae* was 25.1 days (SD ± 22.3; median 17.5 days; range 12.0-28.5). Correlation between between time of stay on ICU and first time of detection of ESBL-*Klebsiella pneumoniae* was significant (R-squared 0.84, $p < 0.001$). Approximately 85% (67/79) of patients received an antimicrobial treatment before the isolation of ESBL-*Klebsiella pneumoniae*, using a mean of 2.82 (SD ± 2.47) different classes of antibiotic *per* patient. Third generation cephalosporins and fluoroquinolones were the most prescribed classes of antibiotics with a percentage of 24.3% and 12.7%, respectively.

Overall 59 ICUs health care workers, out of 141 (41.8%), attended the courses, in particular: 38.9% in ICU A (28/72, of which 27/40 nurses) and 44.9% in ICU B (31/69, of which 27/38 nurses).

Fifty-seven environmental samplings were collected, all of them resulted negative for ESBL-*Klebsiella pneumoniae.* A microorganism with the same pattern of antimicrobial resistance of those involved in the cluster was isolated from the hand of one health-care worker belonging to the ICU A. Moreover, a non ESBL-*Klebsiella pneumoniae* has been isolated from one monitor mobile system belonging to ICU A. RAPD results revealed the presence of a single lineage among all the CTX-M producers, plasmid restriction profile, obtained after digestion with the PstI endonuclease, revealed apparent identity among CTX-M-15-encoding plasmids from all the isolates.

The PFGE pattern showed that all typed strains belonged to the same genotype (Fig. 2), including that isolated from the hands of the health-care worker.

After the implementation of the multimodal program, an evaluation of the infection control intervention was performed. The alcohol based hand rub was actually adopted from 18 March 2006 in ICU A, while ICU B adopted alcohol based hand rub on September 2006 (at the end of the observation period), therefore it has been considered as not having adopted it in this analysis. After its adoption, between 18 March and 30 September 2006, alcohol based hand rub consumption in ICU A has been of about 25 L/1000 patient days, ICU B has not adopted it.

Fig. 2. PFGE of XbaI digested DNA from MDR Klebsiella pneumoniae isolates.

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Tab. II. Comparison of incidence colonization rates/1000 patient days (IDR) before and after the intervention in ICU A (participating) and ICU B (non participating).

	ICU A	ICU B
Pre-intervention IDR (95% CI)	4.50 (2.16-8.28)	4.02 (1.93-7.40)
Post-intervention IDR (95% CI)	1.68 (0.46-4.31)	8.31 (5.08-12.84)

Incidence density rates per 1000 days of hospitalization (IDR) before and after the adoption of alcohol based hand rub by ICU A are showed in Table II; while in the pre intervention period IDR of ICU A was 4.50/1000 patient days (95% CI, 2.16-8.28), in the post-intervention period it fell down to 1.68/1000 patient days (95% CI, 0.46-4.31); moreover, while the IDRs had not been significantly different before the intervention, after the adoption of alcohol based hand rub a significant difference in IDRs has been registered between the two ICUs.

Discussion and conclusions

The epidemiological investigation allows to hypothesize a cross transmission from patient to patient through the hands of the personnel of the same genotype of ESBL-*Klebsiella pneumoniae*. The importance of hand carriage of gram-negative bacteria was shown by Casewell et al. [3] since the late 70s, and has been subsequently reported as the most common in literature for this microorganism [5, 19]. Moreover, experience from outbreaks suggests that targeted surveillance and introduction of strict infection control measures (such as improved hand hygiene and contact precautions) can help control the spread of ESBL-*Klebsiella pneumoniae* [2, 20]. Although environmental sources have been documented in ESBL-*Klebsiella pneumoniae* outbreaks on occasions [21], we think that further elements may support the hypothesis of a transient carriage on the hands of healthcare workers: i.e. the temporal and spatial trend of the outbreak (Fig. 1), and the isolation from the hands of the healthcare worker.

The microorganism spread occurred mainly in critical areas, in fact 84.8% (67/79) of all surveyed patients were admitted there at least for a part of their hospital stay, confirming the major role of ICU in healthcare associated infections [1, 4, 5, 22].

In our experience, the control of the spread of ESBL-*Klebsiella pneumoniae* was carried out thanks to a multimodal approach, which included: administrative support and involvement, educational interventions on isolation guidelines and contact precautions recommendations, introduction of alcohol-based hand rub, clinical audit, surveillance and feedback to health-care workers about laboratory results.

In particular, our findings confirm the important role of active surveillance in critical area to reduce nosocomial spread of multidrug-resistant pathogen strains [5, 23]. Moreover, the educational intervention was effectively turned to a change in behaviour with the actual implementation of alcohol based hand rub, which induced an evident decrease in incidence density rate (IDR) in ICU A. In our opinion the effectiveness of alcohol-based hand rubs, strongly recommended by the World Health Organization (WHO), must be associated with educational interventions about their use and about the importance of hand hygiene [23-25] moreover, we think that the utilization of a proxy, surrogate, measure for its utilization (i.e. product utilization data) could be routinely used to monitor hand washing behaviour change, also the effective measurement of hand washing compliance should be more deeply investigated [26]. The improved hand hygiene compliance during the outbreak period could be linked to the control of the outbreak, in fact, also if we cannot demonstrate a causal link between fall in colonisation and increase in hand hygiene, the inbuilt control group involuntarily offered by the delaying of the intervention in ICU B has given the opportunity to verify an epidemiological association.

In agree with Bryce et al. [27], we believe that the hold collaboration between infection control practitioners (ICPs) and health-care workers, through clinical audit, which includes the review of workplace infection control practices, the assessment of health-care workers' knowledge and the application of infection control principles and feedback of their performance, plays a key role in the effectiveness of infection control programs during outbreaks. In the hospital setting, the statistical significance of the effectiveness of an infection control program is influenced by several confounding factors, as the following: health-care workers' perception of risk, their individual perception of the personal hygiene of patients, and the time of constraints, type of patient contact changes both randomly and continuously in most acute wards, the compliance with and hygiene in any ward vary a lot, reflecting day to day changes in staffing and case mix [28].

Moreover the importance of these factors changes from one hospital to another and we, therefore, believe that a multimodal and specialized approach offers a better opportunity for controlling more factors underlying a good knowledge of the reference context.

In the light of our results, the outcomes achieved, support the effectiveness of the multimodal approach used, however for a successful promotion of evidence-based practices the involvement of local opinion leaders facilitating the cultural modification of health-care workers is essential [29].

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