ORIGINAL ARTICLE

Detection of bacterial pathogens in the hands of rural school children across different age groups and emphasizing the importance of hand wash

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Keywords

Hand hygiene • Bacteria • Children

Summary

Introduction. *Contaminated hands remain the mainstay cause of infection in children. Infections like diarrhoea and pneumonia were found to be common among children who have limited knowledge on the importance of hand wash. The present study was aimed to assess the relationship between the bacterial load sampled from the hands of school children and their routine hand wash practice methods.*
Methods. *Samples were collected from both the hands of 200 rural school children. Bacterial colonies isolated from the swabs were identified by standard microbiological procedures. Questionnaire was provided to gather matrix of routine hand wash practice from the subjects. Proper handwashing technique was demonstrated to children.*
Results. *More than 95% of the children harbored commensal like CoNS and Aerobic spore formers. Other pathogenic bacteria isolated include Acinetobacter species (36.5%), Pseudomonas species 4% (15), Enterococcus species (2%), Klebsiella species (3.5%), Flavobacterium species (1.7%), Escherichia coli (2%), and Enterobacter species (0.75%). It was found that the male children harbored more bacteria in their hands when compared to female population. Bacterial population like Pseudomonas species, Klebsiella species and Enterococcus species were predominant in the hands of children belonging to 7-10 years of age whereas Acinetobacter species, Escherichia coli and Flavobacterium species were slightly higher among 11-15 years of age. This information corresponds to the poor hand washing practices among the children.*
Conclusions. *It can thus be concluded from our study that simple handwashing practices can efficiently reduce the transmission of pathogenic bacteria from our hands and greatly reduce the transmission of infection.*

Introduction

Contaminated hands remain as one of the main sources of potentially pathogenic microorganisms causing respiratory and gastrointestinal tract infections in children [1]. The transmission of pathogenic organisms through feco-oral route was found to be common among children who fail to wash their hands properly. Transmission of these organisms can be prevented by simple handwashing with soap and water [2, 3]. Transient flora like *Escherichia coli, Salmonella species, Shigella species, Klebsiella pneumoniae*, etc., is transmitted through faeco-oral route. Other bacteria in contaminated hands include *Staphylococcus species, Streptococcus species, Pseudomonas species and Bacteroide*s *species* which can also remain as a source of infection in children. Resident commensal flora in the hand usually does not produce any infection and are a part of the normal flora of the hand. A few of the commensal bacteria include *Coagulase Negative Staphylococcus species, Bacillus species, Micrococcus*, *Aerococcus*, *Clostridium species* and *Streptococcus species* [4].
The present study was aimed to assess the relationship between the bacterial load sampled from the hands of school children and their routine hand wash practice methods. The importance of hand wash and the different steps involved in hand wash were also emphasized among the school children.

Materials and methods

This observational study was carried out in the Department of Microbiology, Chettinad Hospital and Research Institute, Kelambakkam for a period of three months. Samples were collected from both the hands of 200 rural school children using sterile saline dipped cotton swabs. The school children were split into two different age groups belonging to 7-10 and 11-15 years of age. Inclusion criteria: Male and female school children of age 7-15. Children with open wound on their hands, nail infection and children with congenital loss of motor activity in their hands were excluded from the study.
Clearance for the study was obtained from the Institutional Human Ethics Committee (IHEC) before beginning the study. Consent was obtained from school authorities and from the parents before collecting the samples from children. Assent from the children was also obtained before collecting the sample. The samples were collected before the lunch time recess of the school.
Samples were collected from both the hands of 200 rural school children (began with the flexor aspect of the wrist which including the palms, thumbs, creases, nail beds and ending with the dorsal aspect) using sterile saline dipped cotton swabs adhering to aseptic procedures and transferred to Microbiology laboratory in Amie's transport medium [1]. Each sample was provided with proper iden-

tification number and other details like age and sex of the child were also noted.

Swabs in the Amie's transport medium transported to the Microbiology laboratory within 1-2 hours were inoculated onto MacConkey agar and blood agar using standard streak plate procedure. The plates were incubated at 37°C overnight and inspected for bacterial colonies and potential bacterial strains were identified by standard microbiological procedures.

The standard microbiological procedures carried out for the identification of these bacterial strains included Gram stain and routine biochemical tests such as catalase test, oxidase tests, Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Triple sugar iron agar reaction, Mannitol fermentation and motility test, Urease reaction etc. [5].

Questionnaire was provided to gather matrix of routine hand wash practice from the subjects [6]. The Questionnaire was translated in the vernacular language for better understanding of the children. The questionnaire was later analysed and tabulated.

The students were given a handwashing kit which also contained a pamphlet showing the various steps in hand washing in the vernacular language. A power point presentation and a short film were shown to the students to make them understand the importance of handwash. Students were randomly selected and were asked to demonstrate the steps in hand washing as per the WHO guidelines (modified) [7]. Posters which implied the importance of hand washing and the steps in hand washing were put up in each classroom.

Statistical analysis

The results were analyzed using IBM SPSS (version 21.0) software. Mann Whitney U test was used to analyze the prevalence of organisms between gender, two age groups and right and left hands.

Results

The hands of 200 school children both right and left were sampled. These children belonged to two age groups 7 to 10 and 11 to 15 years. About 47% of the children were male and 53% of them were female (Fig. 1). A total of 58% of the children belonged to the age group of 7-10 years and 42% of them belonged to 11-15 years (Fig. 2). Organisms isolated from both hands of all of the school children include *Aerobic spore former(ASF)* 99% (397), *Coagulase negative Staphylococcus species* (CoNS) 95.5 % (382), *Acinetobacter species* 36.5 % (146), *Pseudomonas species* 4% (15), *Enterococcus species* 2% (8), *Klebsiella species* 3.5% (14), *Flavobacterium species* 1.7% (7) and *Escherichia coli* 2% (8), *Enterobacter species* 0.75% (3) (Fig. 3).

Most of the organisms isolated from school children were equally distributed in both the hands except for *Acinetobacter species* that was found to be higher in the left hand. Other Gram negative organisms like *Pseudomonas species, Flavobacterium species, Escherichia coli* and *Klebsiella species* were found to be slightly higher in right hand (Fig. 4).

Commensal bacteria like aerobic spore former and CoNS were found to be significantly higher in the hands of female children ($p = 0.32$) whereas *Acinetobacter species*, *Pseudomonas species*, *Enterococcus species*, *Escherichia coli* and *Flavobacterium species* were found to comparatively higher in the hands of male children ($p < 0.05$) (Fig. 5).

Pseudomonas species, Klebsiella species, Enterococcus species were found be significantly higher in the hands of children belonging to 7-10 years of age ($p = 0.01$). Other organisms like *Acinetobacter species* ($p = 0.00$), *Enterobacter species* ($p = 0.039$), *Escherichia coli* ($p = 0.001$) *and Flavobacterium species* was found be predominant in the hands of children belonging to 11-15 years of age. Commensal bacteria like *CoNS* and *Aerobic spore former* were equally predominant in the hands of children belonging to both age groups (Fig. 6).

In the age group of 7-10 years, 36% of the students responded that they wash their hands 1-5 times a day and about 63% of the students responded that they wash their hands 6-10 times a day. Only one student has responded as never. About 89% of the children have given the rea-

Fig. 1. % of male and female rural school children participated in the study.

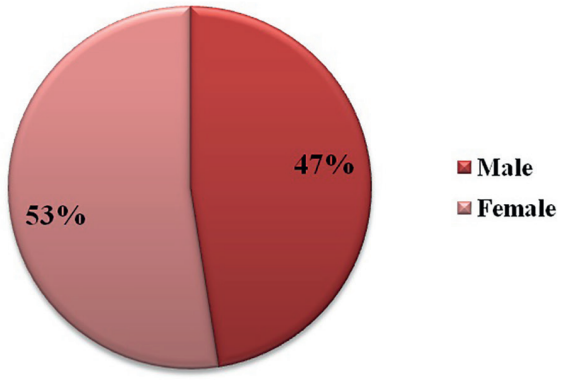


Fig. 2. % of children with two different age groups participated in the study.

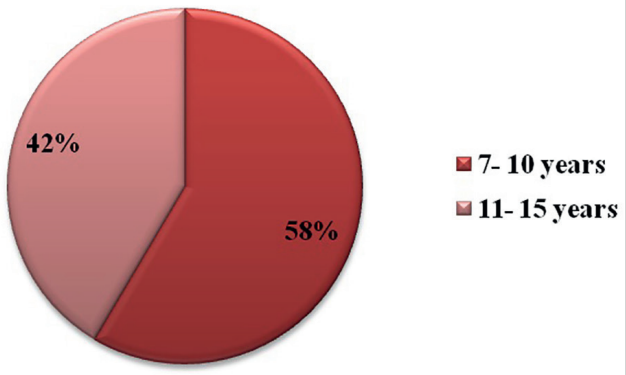


Fig. 3. Total organisms isolated from all children participated in the study.

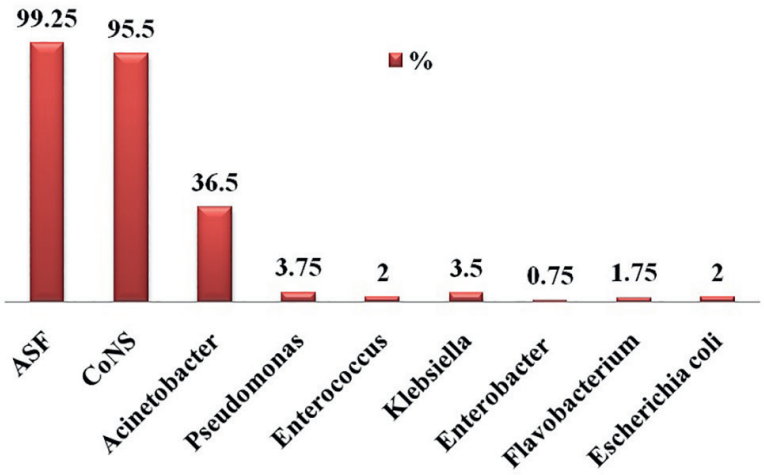
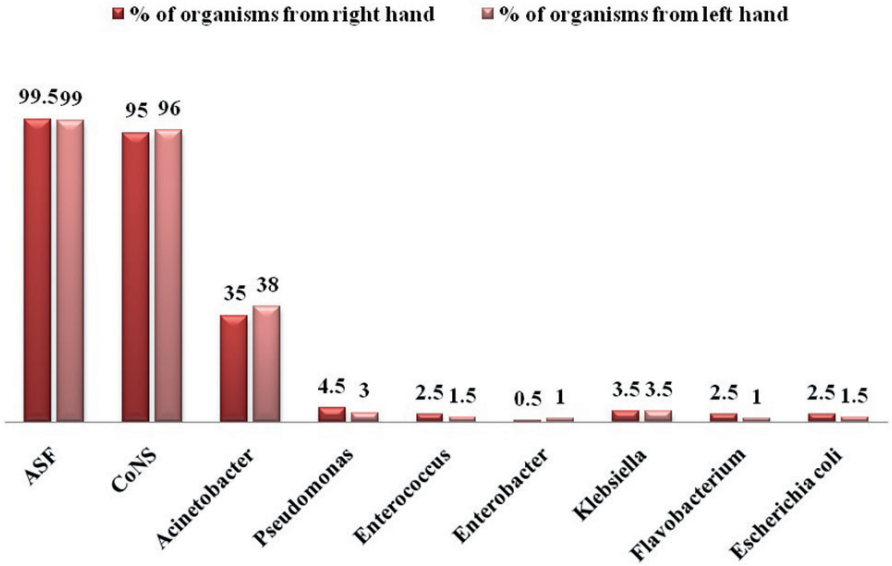


Fig. 4. Total organisms isolated from both hands.

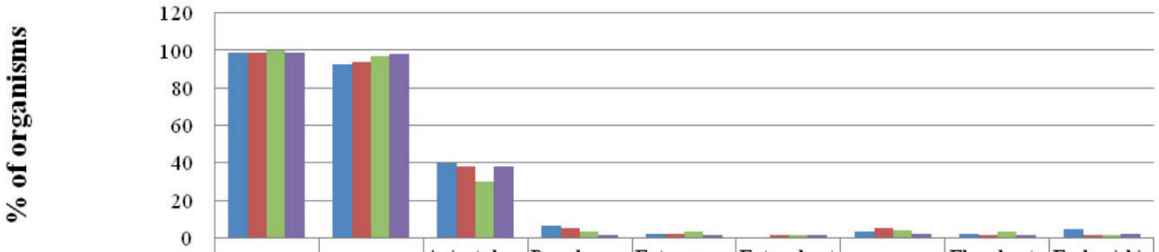


son for skipping handwashing as the wash sinks in the schools are placed in a far away location with no easy accessibility. About 72% of the children were aware that improper handwashing can result in the transmission of disease and 28% of the children were unaware of the fact that improper hand washing can cause disease (Tab. I).
In the age group of 11-15 years, 18% of the students responded that they wash their hands 1-5 times a day and about 82% of the students responded that they wash their hands 6-10 times a day. About 92% of the children have given the reason for skipping handwashing as the wash sinks in the schools are placed in a far away location with no easy accessibility. About 99% of the children were aware that improper handwashing can result in the transmission of disease and 1% of the children were unaware of the fact that improper hand washing can cause disease (Tab. II).

Discussion

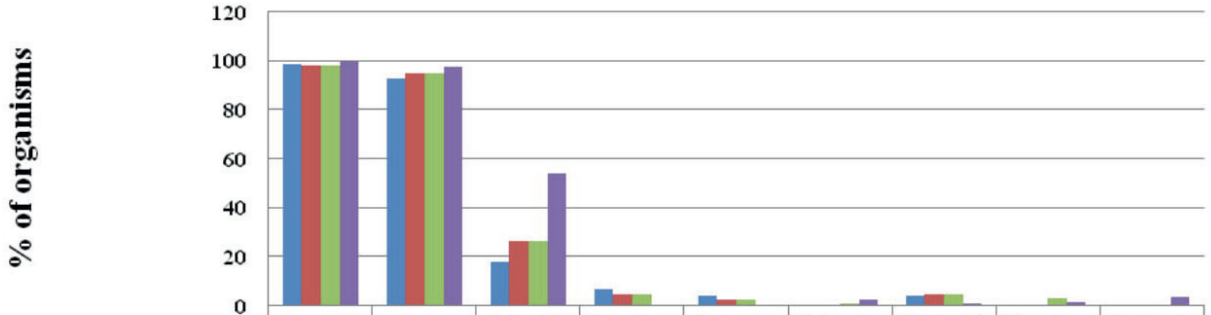
In the present study most of the rural school children harboured commensal flora like *Aerobic spore formers* and *Coagulase negative Staphylococcus species (CoNS)*. This was consistent with many studies. However, 2% to 36% of the children harboured pathogenic bacteria like *Acinetobacter species*, *Pseudomonas species*, *Klebsiella species*, *Flavobacterium species* and *Enterobacter species*. These bacteria can occur as opportunistic pathogens and can cause community acquired infections.
In this study, male children were colonized with relatively higher bacterial population when compared to female children. But the distribution of bacterial flora in right and left hand was found to be inconsistent. Organisms like *Coagulase negative Staphylococcus species (CONS)*, *Acinetobacter species* and *Enterobacter species* were found to be comparatively high in left hand.

Fig. 5. Organisms isolated from right and left hands of male and female children.



	ASF	CoNS	Acinetobacter	Pseudomonas	Enterococcus	Enterobacter	Klebsiella	Flavobacterium	Escherichia coli
Male Right hand	98.95	92.63	40	6.32	2.11	0	3.16	2.11	4.21
Male Left hand	98.95	93.68	37.89	5.26	2.11	1	5.26	1.05	1.05
Female Right hand	100	97	30	2.8	2.8	1	3.8	2.8	0.95
Female Left hand	99	98	38	0.95	0.95	1	1.9	0.95	1.9

Fig. 6. Organisms isolated from right and left hands of children belonging to two age group.



	ASF	CoNS	Acinetobacter	Pseudomonas	Enterococcus	Enterobacter	Klebsiella	Flavobacterium	Escherichia coli
7-10 years Right hand	99	93	18	7	4	0	4	0	0
7-10 years Left hand	98	94.8	26.5	5	2.5	0	5	0	0
11-15 years Right hand	98	94.8	26.5	5	2.5	1.2	5	3	0
11-15 years Left hand	100	97.6	54	0	0	2.4	1.2	1.7	3.6

Bacterial population like *Pseudomonas species, Klebsiella species and Enterococcus species* were predominant in the hands of children belonging to 7-10 years of age whereas *Acinetobacter species, Flavobacterium species* and *Escherichia coli* were predominant among 11-15 years of age. This was concordant with the study report of Ghimire et al. where maximum isolates were from children belonging to 10-12 years of age [6].

The prevalence of *Acinetobacter species* was high when compared to the study by Ghimire et al. who report *Acinetobacter species* isolation rate of only 2.5% [6]. According to the report given by Matthieu Eveillard et al., *Acinetobacter species* remains as an important cause of pneumonia in the community and also associated with wound infections and cause life-threatening infections in immunocompromised individuals as emergence of multidrug resistance is very common among these organisms [8].

Other Gram negative bacilli isolated in our study were *Pseudomonas species* 4% (15) *Klebsiella species* 3.5% (14), *Flavobacterium species* 1.7% (7) and *Escherichia coli* 2% (8) and *Enterobacter species* 0.75% (3). The colonization rate of these organisms was found to be comparatively low when compared to the study reports of Ghimire and Ray et al. [1, 6].

In this study about 1.7% of the children harboured *Flavobacterium* in their hands. This bacterium is ubiquitous and is commonly present in moist environments and tap water which can remain as a common source of contamination [9]. In our study *Escherichia coli* was present in 2% of the children. *Enterotoxigenic E. coli* remains as a major cause of diarrhea in young

Tab. I. Response to questionnaire by children belonging to 7-10 years of age.

S. No	Questions	Always (%)	Sometimes (%)	Never (%)
1	I wash my hands before meals	84	8	8
2	I wash my hands after meals	85	7	8
3	I wash my hands after using the restroom	85	8	7
4	I wash my hands before going to bed	26	54	20
5	I wash my hands after using public transportation	30	60	10
6	I wash my hands after waking up in the morning	75	10	15
7	I wash my hands after touching animals	76	11	14
8	I wash my hands only if they are soiled	78	11	11
9	I wash my hands after blowing my nose/sneezing	75	9	16
10	I wash my hands after touching the garbage	79	9	12
11	I wash my hands before touching sick people	70	13	17
12	I wash my hands after touching sick people	78	8	14
13	Do you use any soaps/ disinfection for hand washing?	71	11	18
14	Is there any wiping material available in restrooms?	72	6	22
15	Is there always soap and water available to wash your hands?	79	9	12

Tab. II. Response to Questionnaire by children belonging to 11-15 years of age.

S. No	Questions	Always (%)	Sometimes (%)	Never (%)
1	I wash my hands before meals	87	10	3
2	I wash my hands after meals	95	4	1
3	I wash my hands after using the restroom	93	6	1
4	I wash my hands before going to bed	35	60	5
5	I wash my hands after using public transportation	37	60	3
6	I wash my hands after waking up in the morning	85	10	5
7	I wash my hands after touching animals	90	6	4
8	I wash my hands only if they are soiled	91	7	2
9	I wash my hands after blowing my nose/sneezing	85	9	6
10	I wash my hands after touching the garbage	92	4	4
11	I wash my hands before touching sick people	85	7	8
12	I wash my hands after touching sick people	90	8	2
13	Do you use any soaps/ disinfection for hand washing?	91	8	1
14	Is there any wiping material available in restrooms?	80	12	8
15	Is there always soap and water available to wash your hands?	82	16	2

children. About 4% and 3.5% of the isolates of the children harbored *Pseudomonas species* and *Klebsiella species* which could serve as a source of lower respiratory infections like pneumonia [10]. Thus contaminated hands of children can be a major source of infection for individuals harbouring some underlying medical ailments.

Children belonging to 11-15 years of age had more awareness about handwashing when compared to children belonging to 7-10 years of age. Majority of the children from both the age groups skipped handwashing as the wash sinks were placed in a far away location.

In our study, we gathered a matrix to assess the handwashing practice among children and of the school children have better awareness about the importance of handwashing. But a study by Garg et al., promoted the importance of handwashing among children and concluded with pre and post intervention tests to assess the knowledge of the students about hand washing [11].

Conclusions

The present study shows that the hands of school children harbored a variety of pathogenic organisms which can cause serious diseases like respiratory tract infections and gastro intestinal infections which are possibly fatal if not attended to. Lack of a proper hand washing model, hand washing material like clean water, soap, wiping material and the presence of hand wash sinks at inaccessible locations were a few of the reasons for failure of handwashing practice in schools. Provision of these materials can bridge the gap between proper hand washing techniques and the prevention of spread of infection. It was thus very clear from our study that proper hand washing practices can prevent the spread of diseases. It is encouraged to educate the children on the importance of hand wash, as simple handwash procedure can help in controlling a variety of diseases and they should see them as an important action in their daily life rather than viewing it as a normal activity. Educating the students should not only stop with the schools. We

must also make sure that the information taught to the students is not only followed in schools but is also followed at home and thus can ultimately reach the society to understand the true benefits of hand washing.

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Conflict of interest statement

None declared.

Authors' contributions

RV, APS and PS designed the study. RV and APS collected and processed the samples and analyzed the results. RV, APS and PS participated in article revision. All the authors of this manuscript gave approval.

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