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RESEARCH ARTICLE

THUMB IMPRESSION ANALYSIS: ISOLATION AND EVALUATION OF ANTIBIOTIC RESISTANCE
PROFILES OF THE BACTERIAL STRAINS

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ABSTRACT

Millions of people around the globe suffer from various infectious diseases per annum. Extensive part of these infections is hand acquired infections. World health organization suggests hand hygiene as a primitive precaution focus to decrease the infection rates. We evaluated various aspects related to the occupational behavior and prioritization regarding hand hygiene measures among the university students, teachers, laboratory staff and canteen workers. Gram positive cocci were found in abundance followed by Gram positive rods. The antibiotics resistance profile showed that the isolated bacterial strains were mainly sensitive to streptomycin, whereas, majority of the isolates were resistant to cefixime-a third generation cephalosporin. Methicillin resistance was also found common among *Staphylococcus* spp. The study indicated the resistance spread among the opportunistic normal flora and the role of unhygienic hands in their transmission.

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INTRODUCTION

Although, the discovery of antibiotics predicted the ultimate control of microbial infections but this goal could not be successfully achieved because of the rapid emergence of resistance among pathogenic micro-bugs. In general, the frequent use of antimicrobial agents causes the selective pressure which led to the resistance (Jones and Pfaller, 1998). The pathogenic microbes and their antibiotic sensitivity may change from time to time and place to place. Thus, understanding prevalent antibiotic resistance pattern of the common pathogenic bacteria in a particular region is particularly useful in clinical practices (Rajat *et al.*, 2012). Various epidemiological studies manifested the spread of antibiotic resistance among microbial pathogens (Koneman *et al.*, 2006). The human microbiota warrants special attention as perhaps being the most accessible reservoir of resistance genes due to the high likelihood of contact and genetic exchange with potential pathogens (Penders *et al.*, 2013). Hands are the most important part of the body with respect to the exposure and spread of bacteria. Particularly, in health care settings and clinical lab setups where surfaces are often contaminated, the hands of the staff and lab personnel do serve as a vector for cross contamination (Ayçiçek *et al.*, 2004).

Hand washing with a sufficient amount of water and soap removes more than 90% of superficial temporary infective factors (Nero *et al.*, 2011). On the other hand, it has also been reported that use of these antibacterial soaps and hand washings may serve as an important factor in spreading the resistance among bacteria (Aiello *et al.*, 2007). Hand skin flora can be categorized into resident and transient flora. Resident flora generally inhabits the substratum corneum as well as superficial layers of the skin whereas; transient flora on hands is generally acquired by poor hygiene that may further result in cross infections. However, the spread of transient flora depends on a number of factors such as the type of species present, the microbial load on the surfaces and moisture content (Patrick *et al.*, 1997). Persistent colonization with pathogenic microbes such *S. aureus*, Gram negative bacilli and yeast strains has been frequently noticed on the hands of health care workers (Allegranzi and Pittet 2009).

Thus, the aim of the present study was to evaluate the hand hygiene status of the university attending personnel such as students, teachers, lab staff and food handlers and to determine the prevalence of antimicrobial resistance among the bacterial strains isolated from their hand surfaces.

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MATERIAL AND METHODS

Thumb Impression Analysis

Thumb impressions of different groups of individuals were taken aseptically on nutrient agar plates. These groups were comprised of teachers, students, food handlers and laboratory assistants of University of Karachi. Afterwards, these media plates were incubated at 37°C for 24 hours. Next day, morphologically distinct colonies were selected and purified by further streaking on nutrient agar slants.

Identification of the isolates

The isolated strains were identified on the basis of their morpho-cultural and biochemical characteristics. Gram positive bacteria were maintained on mannitol salt agar plates whereas, MacConkey's agar plates were used to maintain Gram negative isolates.

Antibiograms

The antibiotic sensitivity profile of isolates was determined by Modified-Kirby Bauer disc diffusion method according to CLSIs guidelines. Freshly grown overnight cultures of the isolates were taken and the turbidity of these broths was compared with 0.5 McFarland index. Following bacterial lawn preparation on Mueller Hinton Agar, different antibiotic discs (Streptomycin, Penicillin, Methicillin, Cefixime, Novobiocin and Bacitracin) were placed and these plates were incubated at 37°C for 24 hours. Next day, the zone of inhibition was recorded and interpreted as sensitive/resistance.

RESULTS

A total of 439 colonies were isolated and identified after taking the thumb impression of the targeted groups including teachers, students, lab staff and food handlers (Table 1). It was observed that the bacterial count was varied from person to person as per the nature of their job and hygienic conditions. A significantly high bacterial load was found on the hands of canteen workers followed by laboratory staff whereas, the lowest bacterial load was noticed on the hands of girls wearing gloves.

Table 1. Isolation of bacteria from different groups

Group	Number of colonies isolated
Teachers	86
Students	78
Laboratory Assistants	135
Canteen workers	140

As shown in Fig 1, majority of the isolates were gram positives including 29.62% Gram positive cocci in bunches (*Staphylococcus* spp), 25.92% Gram positive rods (*Bacillus* spp) and 18.5% gram positive chains (*Streptococcus* spp), However, 11.11% strains were observed as gram negatives (*E.coli*). Antibiotic resistance profiles of the isolates are summarized in table 2. According to the results, the gram positive cocci were found more resistant to the (tested) antibiotics when compared with gram positive rods. Most of

the isolates were found sensitive to streptomycin while, the majority of the isolates showed resistance to cefixime- a third generation cephalosporin (Fig. 2). Methicillin resistance was observed in 55.4% *Staphylococcus* spp. suggesting the presence of MRSA as a member of normal skin flora. The resistance towards penicillin was observed in 85% of *Staphylococcus* spp. and 54.5% in *Streptococcus* sp. The *Bacillus* sp. were found comparatively more susceptible to tested antibiotics.

Table 2. Percentages of the Isolates Resistant to the Tested Antibiotic

Antibiotics	Percent of the isolates become resistant				
	<i>Bacillus</i>	<i>E.coli</i>	<i>Staphylococcus</i>	<i>Streptococcus</i>	Others
Streptomycin	3.44	2.65	3.44	3.44	4.6
Penicillin	10.33	100	85	54.5	25.6
Methicillin	28.8	52.5	45.7	68.5	55.4
Cefixime	35.8	45.9	85.5	75.9	89.0
Novobiocin	26.5	38.8	58.6	49.8	48.5
Bacitracin	40.5	35.6	42.6	44.5	38.5

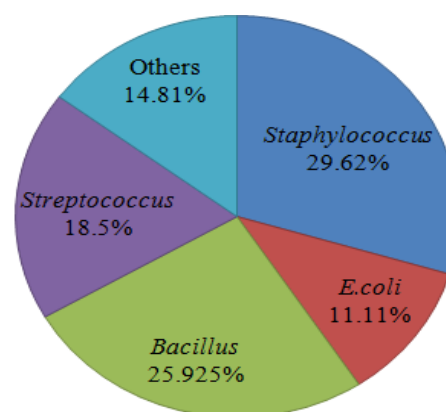


Fig. 1. Percentage of the isolates from hand.

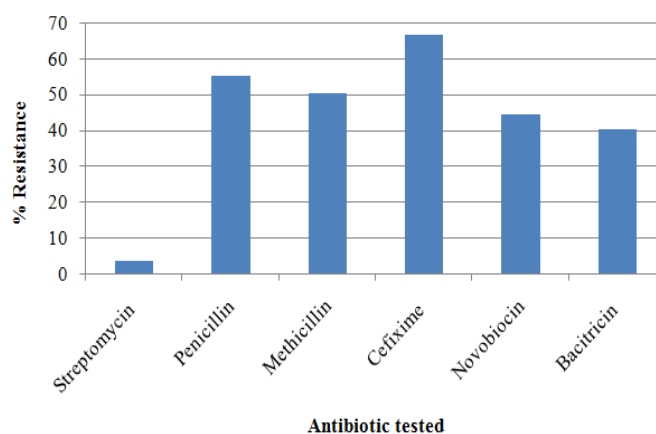


Fig. 2. Percentage of Resistance to tested Antibiotics

DISCUSSION

Surveillance programs have turned out to be a well-accepted requirement to possibly decrease the rate of continuously emerging antibiotic resistance among pathogenic micro bugs, Nevertheless, combined efforts at local, national, and international levels are required. Furthermore, Multicenter

surveillance programs monitoring should also be introduced for understanding the relative importance of risk factors, the evolution of resistance over time as well as for the development and assessment of preventive measures (Guzmán-Blanco *et al.*, 2000). The predominant causative agents for infections include Gram negative rods and *S.aureus*, Multidrug resistant coagulase-negative Staphylococci are of a particular concern as the incidence of infections caused by these organisms is increasing and such infections are often nosocomial acquired. In the present study, it was noticed that the level of awareness also influences the hygienic status of the personnel as teachers and students (graduating from biological sciences) displayed a good hygienic status and less bacterial load. The highest bacterial load was found on the hands of food handlers and laboratory staff, indicting their exposure, lack of awareness and hygiene status of hands. Although, most of the isolated bacteria strains were the expected ones as the normal flora of the skin, but their increased resistance towards antibiotics is a point of concern in the current study as 85% and 55.4% of *Staphylococcus* spp were resistant to Penicillin and Methicillin respectively. Our findings are in consonance with Okeke *et al.* (2005) who isolated 88.7% Penicillin resistant *Staphylococcus* spp whereas, Kapil *et al.* (2015) observed 45% methicillin resistant *Staphylococcus aureus* sp from the hands of health care workers. It is also important to note that the majority of the isolates particularly *Staphylococcus* and *Streptococcus* spp. were resistant to cefixime which is a 3rd generation cephalosporin usually given for treating the upper respiratory tract infections. Although, it is already reported that cefixime is ineffective in controlling the *Staphylococcus* and *Streptococcus* infections (Schito *et al.*, 2002) but the emerging resistance to this antibiotic in other groups of bacteria is alarming. Thus, the study necessitates the arrangement of awareness seminars for lab workers and food handlers in the university settings with a particular focus on the significance and maintenance of good hygiene status.

Conclusion

Present study demonstrates that the hand hygiene plays an important role in transmission of cross infections and it is necessary to generate evidences manifesting the role of good hygienic status in reducing the bacterial load on the hands as a tool to educate the masses and reduce the frequency of community acquired infections. Prevalence of bacterial resistance to the commonly prescribed antibiotics should also be a particular focus and it demands the training of the primary health care practitioners and to discourage the trends of indiscriminate use of antibiotics and self-medication practices in the country.

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