ORIGINAL ARTICLE

ANTIBIOTIC RESISTANCE PROFILE: ISOLATION AND CHARACTERIZATION OF CLINICAL ISOLATES OF STAPHYLOCOCCI FROM PATIENTS WITH COMMUNITY-ACQUIRED SKIN INFECTIONS

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ABSTRACT
Although most bacterial infections of the skin bear out to be minor in nature, a few such dermatologic entities are major, to the spot of yet being fatal. The mortality rate is usually up to 30% to 50% and depends upon the type of infection, original disease, and resistant type. In this study hundred and five bacterial strains were isolated from skin wounds, burns and acne patients from hospitals at different locations in the cosmopolitan city of Karachi. These bacterial strains were identified by conventional methods. Seventy two percent (72%) of total isolated organisms were found to be *Staphylococcus aureus* while the remaining thirty three percent (33%) were *Staphylococcus epidermidis.* The antibiotic resistance of identified organisms was carried out by disc-diffusion method with commercially available disc of five antibiotics having different mode of actions such as cell wall synthesis inhibitors, membrane permeability alternatives and DNA synthesis inhibitors. *Staphylococcus aureus* show more resistant to these antibiotics as compared to *Staphylococcus epidermidis.* The most effective antibiotic for *Staphylococcus aureus* is vancomycin showing 80.5% efficacy, then methicillin with 68.0% efficacy, erythromycin with 55.6% efficacy, novobiocin with 54.1% efficacy and then bacitracin with 25.0% efficacy. The most effective antibiotic for *Staphylococcus epidermidis* is methicillin showing 84.8% efficacy, then vancomycin with 81.2% efficacy, novobiocin with 63.6% efficacy, erythromycin with 42.4% efficacy and then bacitracin with 27.8% efficacy.

Keywords: *Staphylococci,* skin infection, antibiotics, antimicrobial and resistance.

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INTRODUCTION

Compared with the very complicated bacterial flora establish in the gastrointestinal tract or in the oral cavity, the normal flora of the skin is moderately simple and usually consists of a few aerobic and anaerobic bacteria (Higaki *et al.,* 1999). Even though most bacterial infections of the skin provide evidence to be unimportant in nature, a few such dermatologic entities are momentous, to the point of even being fatal. Their course can be enormously rapid and can guide to dreadful complications. The mortality rate is usually up to 30% to 50% and depends upon the nature of infection, underlying disease, and immune status. Patients suffering them usually need to be hospitalized, sometimes in intensive concern or burn units. The two life-threatening skin infections which are most commonly experienced are toxin-mediated staphylococcal and streptococcal disorders; one may possibly have common characteristics to the other (Yamasaki *et al.,* 2006; Marina *et al.,* 2005; Schmidt *et al.,* 2005). *Staphylococcus aureus* has emerged as one of the mainly important human pathogens, and has over the past numerous decades, been a leading foundation of hospital and community-acquired infections (Shittu *et al.,* 2006; Loffler *et al.,* 2005; Johnsson *et al.,* 2004). Staphylococcal infections give rise to a wide spectrum of symptoms and diseases in humans. *Staphylococcus aureus* is well characterized and known to have a diverse arsenal of virulence factors that causes a prominent inflammatory response. (Augustinsson *et al.,* 2004; Davison *et al.,* 2000). *Staphylococcus aureus* skin infections were classified as primary or secondary. Primary infections were those occurring on apparently normal skin, and mainly comprised impetigo, ecthyma, folliculitis, furuncles, sycosis barbae, cellulitis, abscesses, paronychia and whitlows. Secondary infections were those arising in damaged skin (traumatized skin, or a pre-existing skin disease) (Giudice *et al.,* 2006; Simou *et al.,* 2005). Coagulase-negative Staphylococcus (CNS) which is also termed as *Staphylococcus epidermidis* is a mainly important component of the normal skin flora. From the time of the early 1980s, *Staphylococcus epidermidis* has also emerged as an important pathogen (Vandecasteele *et al.,* 2003; Kolawole *et al.,* 1997). *Staphylococcus epidermidis* behave in a very different way, it is an essential commensal in the normal bacterial flora of human skin, but, in a clinical setting, it is important as the main pathogen in foreign body infections (Augustinsson *et al.,* 2004). Since the introduction of antimicrobials, bacteria have developed mechanisms for resisting the effects of antibiotics. The emergence of multidrug resistance in Gram-positive bacteria (pneumococci, enterococci and staphylococci) is a particularly important development. Perhaps the pathogen of greatest concern is Staphylococcus aureus, because of its intrinsic virulence, its ability to cause an array of lifethreatening conditions, and its capacity to adapt to different environmental conditions (Karchmer, 2006; Kern, 2006). The development of modern antibiotics has improved the treatment of cutaneous bacterial infections so much that today most dermatoses caused by bacteria can be treated effectively. Unfortunately, however, the indiscriminate use of antibiotics in some parts of the world in both human and veterinary medicine has led to the emergence of resistant strains of bacteria (Vayalumkal *et al.,* 2006; Capitano *et al.,* 2003; Veien, 1998). Currently, a clinically significant number of staphylococcal species that infect humans and domestic animals exhibit some degree of antimicrobial resistance. In human medicine, methicillin resistance (MR) in *S. aureus* strains has contributed to the scope of multidrug resistance since the early 1960s (Morris *et al.,* 2006; Khawcharoenporn *et al.,* 2006; Segreti, 2005). The aim of the current study was based on the isolation, identification and antibiotic resistance pattern of about hundred and five clinical isolates of skin infections.

MATERIAL AND METHOD

Collection of Samples

Staphylococcus isolates were obtained from septic wounds and burns patients undergoing injury dressing at different hospitals. Wound exudates were obtained from the infected sites of each patient with sterile cotton swabs and applied to freshly prepared slants of nutrient agar and mannitol salt agar (Oxoid). The cultures were then transferred to the laboratory where they were incubated at 37^{o}C for 24 hrs (Kolawole *et al.,* 1997; Huys *et al.,* 2002).

Bacterial Isolates, Culture Media and Species Identification

Colonies growing on slants were streaked on top of freshly prepared plates of mannitol salt agar and incubated again. Primary characterization of isolates was based on the Gram stain, morphological and cultural characteristics. Identification also includes growth on different media including Nutrient agar and Brain Heart Infusion agar, fermentation on Mannitol Salt agar (Oxoid). Catalase and coagulase tests were also performed for biochemical characterization (Udo *et al.,* 2006).

Maintenance of Clinical Isolates

Stock cultures were maintained in vials by growing the skin isolates in 3 ml nutrient broth and next day overlaying with 3 ml 40% glycerol. Vials were than freezed at -70^{o}C (Gul *et al.,* 2004).

Determination of Antibiotic Resistance Profile

Skin isolates were subjected to antibiotic resistance screening by disk diffusion method. For this purpose inocula were prepared by diluting overnight cultures in sterile sodium chloride (0.9%) suspension and then match with the Macfarlane index. Bacterial suspensions were

Table 1: Antimicrobial susceptibility of 105 Staphylococcus species and reference values for the disc diffusion method

	Bacitracin			Methicillin			Erythromycin			Vancomycin			Novobiocin		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Inhibition (mm)	13	9-12	8	14	10-13	9	22	14-22	13	12	10-11	9	22	18-21	17
Distribution of Susceptibility (%)	30			78			54			83			60		

S, susceptible; I, intermediately susceptible; R, resistant.
*CASFM (Frankreich), NCCLS (USA), DIN58940-2 Bb 1: 2000-2001, BgVV (German Federal Institute for Consumer Health Protection

Table 2: Total percentage efficacy of different antibiotics among skin infection isolates

Total No. of isolates (105).

Antibiotics	Disc code	Sensitive	Resistant	Efficacy (%)
Methicillin	Met	78	27	74.2
Bacitracin	Bac	30	75	28.5
Vancomycin	Van	83	22	79.0
Novobiocin	Nov	60	45	57.1
Erythromycin	Ery	54	51	51.4

then plated onto Mueller-Hinton agar (Oxoid) and the commercially available antibiotic discs were placed on lawn of culture and the plates were incubated over night at 37^{o}C (Hoeger, 2004; Veronica *et al.,* 2006). Sensitivity, intermediate sensitivity, and resistances were determined by the zone of complete growth inhibition around each disk according to reference standards (table 1). The following antibiotic discs were used: methicillin (10 ug), bacitracin (15 ug), vancomycin (30 ug), novobiocin (30 ug) and erythromycin (15 ug).

RESULTS AND DISCUSSION

The present study was designed to identify the susceptibility or the resistance profile of the multi-drug resistant isolates from community-acquired skin infections. For this purpose hundred and five clinical isolates of Staphylococci from different clinical laboratories of Karachi were isolated and identified by conventional methods. Identification of the causative organism and its susceptibility to antimicrobials is important, so that proper drug is chosen to treat the patient in early stages (Gul *et al.,* 2004). The frequency of *Staphylococcus aureus* causing skin infection is more than *Staphylococcus epidermidis* (Nishijim *et al.,* 2003). Statistical analysis of the data showed that seventy two percent (72%) of total isolated organisms from skin infections were found to be *Staphylococcus aureus* while the remaining thirty three percent (33%) were *Staphylococcus epidermidis.* Staphylococci are the representative of family Micrococcaceae, and most Staphylococcus species are common inhabitants of the skin surface (Higaki *et al.,* 1999). Previous studies have also suggested that *Staphylococcus aureus* is the most frequent etiological agent causing community and hospital acquired skin infections (Nishijim *et al.,* 2003; Schmidt *et al.,* 2005; Morris *et al.,* 2006).

All the isolates were screened for drug resistance profile by disc-diffusion method with commercially available disc of five antibiotics, that is, methicillin (cell wall synthesis inhibitors), novobiocin (membrane permeability alternatives), bacitracin (cell wall synthesis inhibitors), erythromycin (DNA synthesis inhibitors) and vancomycin (cell wall synthesis inhibitors). Table 2 indicates the resistance level against commonly used antibiotics in skin infections. Nearly all the isolates (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were found to be sensitive against most of the antibiotics whereas *Staphylococcus aureus* show more resistance as compare to *Staphylococcus epidermidis* fig. 1 and fig. 2. Resistance to bacitracin, methicillin and vancomycin in gram positive organisms is often due to B-lactamases which are unaffected by exposure of the bacterium to the potential drugs (Shafran, 1990). It has been argued that there is a direct relation between the antibiotic used and the frequency and kinds of antibiotic-resistant strains in human beings (Gales *et al.,* 2002). The resistance to antimicrobial agents can readily be transferred among bacteria by transmissible elements/plasmids (Neu, 1994). These resistant organisms can pass their resistance genes to their offspring by replication or to related bacteria through conjugation (Tomasz, 1994). Epidemiological studies have suggested that antibiotic resistance genes emerge in microbial populations within 5 years of the therapeutic introduction of an antibiotic (Chakrabarty *et al.,* 1990). Further, the antibiotic resistance genes (found in human and animal isolates) could have originated in

the industrial microbes that have used in the production of antibiotics (Web *et al.,* 1993).

Table 3: Percentage effectiveness of different antibiotics against *Staphylococcus aureus* and *Staphylococcus epidermidis.*

Antibiotics	*taphylococcus aureus* (%)	*Staphylococcus epidermidis* (%)
Methicillin	68.0	84.8
Bacitracin	25.0	27.8
Vancomycin	80.5	81.2
Novobiocin	54.1	63.6
Erythromycin	55.6	42.4

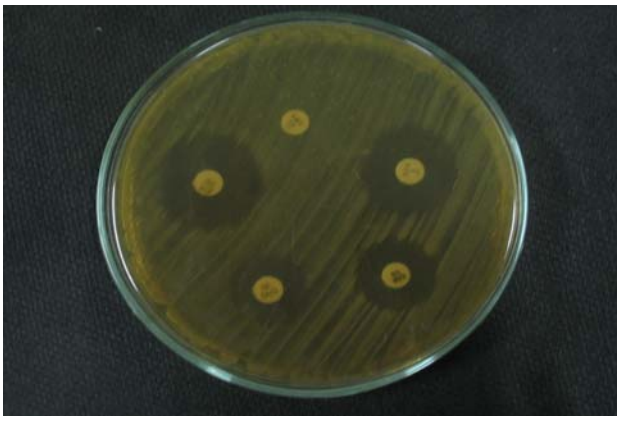

Fig 1: Results of antimicrobial susceptibility patterns of five different antibiotics against *Staphylococcus aureus.*

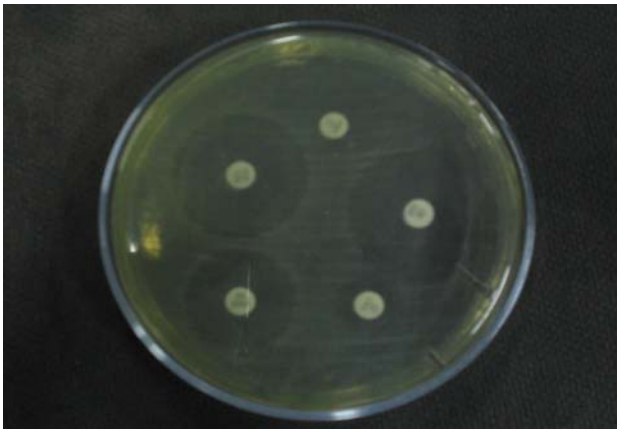

Fig 2: Results of antimicrobial susceptibility patterns of five different antibiotics against *Staphylococcus epidermidis.*

In our studies, the most effective antibiotic for Staphylococcus *aureus* is vancomycin showing 80.5% efficacy, then methicillin with 68.0% efficacy, erythromycin with 55.6% efficacy, novobiocin with 54.1% efficacy and then bacitracin with 25.0% efficacy. Surveillance studies indicate that resistance to b-lactam antibiotics among *Staphylococcus aureus* continues to increase (Karchmer, 2006). Previous investigations suggest that the skin of patients with atopic dermatitis has increased binding avidity for *Staphylococcus aureus* (Yamasaki *et al.,* 2006). The susceptibility of *S. aureus* strains to erythromycin, methicillin and vancomycin was generally high while that to bacitracin and novobiocin was low. This suggests that the penicillinase-resistant anti-staphylococcal agents should be selected as a first choice to treat these infections ((Higaki *et al.,* 1999). Recently, the frequency of isolation of MRSA from skin infections has been increasing (Shittu *et al.,* 2006). Our results, however, were not compatible with the report of Shittu *et al.* regarding the susceptibility of *S. aureus* to some antimicrobial drugs, and this apparent conflict will need to be evaluated in more detail using many more clinically isolated strains. Among the antibiotics tested for *Staphylococcus epidermidis*, methicillin showed the highest rates of efficacy, that is 84.8%, then vancomycin with 81.2% efficacy, novobiocin with 63.6% efficacy, erythromycin with 42.4% efficacy and then bacitracin with 27.8% efficacy (table 3). Present results are in close agreement with Higaki *et al*. In spite of growing concern about rising resistance rates, erythromycin is still one of the mainstays of therapy for this indication. Recent reports from worldwide antimicrobial surveillance programmes indicated that between 49 (USA, 16), 38 (Europe, 17) and 24% (Asia, 18) of all S. aureus strains are now resistant to erythromycin (Holden *et al*, 1998).

Why certain bacteria more commonly infect the skin of particular species is still speculative. Noble provided good evidence that different staphylococcal species have evolved together with their hosts (Noble, 1993). Results of antimicrobial susceptibility testing and total percent efficacy of different antibiotics among skin isolates are summarized in fig. 3 and fig. 4. Akiyama *et al.,* recommended that treatment of staphylococcal skin infections should involve appropriate antibiotics to prevent the emergence of antibiotic resistance (Akiyama *et al*, 1997). Antimicrobial agents currently used to treat resistant strains of Gram-positive bacteria have various clinical and microbiological limitations. For example, tolerability can be problematic with vancomycin (Sivagnanam *et al*, 2003). Topical therapy of bacterial skin infections offers potential advantages over systemic therapy such as absence of systemic side-effects and reduced cost. However, recurrence rates are more than twice as high in topically treated skin infections. Furthermore, topical therapy promotes the development of resistant strains (Shah *et al.*, 2003).

CONCLUSION

The present study demonstrated that the Staphylococci are important pathogens in the context of skin infection and health care and the emergence of community-acquired skin infections isolates is a worrying development.

Numerous studies have demonstrated higher resistance to most of the antibiotics used for the treatment of skin infections. In accumulation, the appearance of pathogens with resistance to various antimicrobial agents indicates a growing need for new antimicrobial agents, with novel modes of action, for use in the treatment of serious Staphylococcal infections.

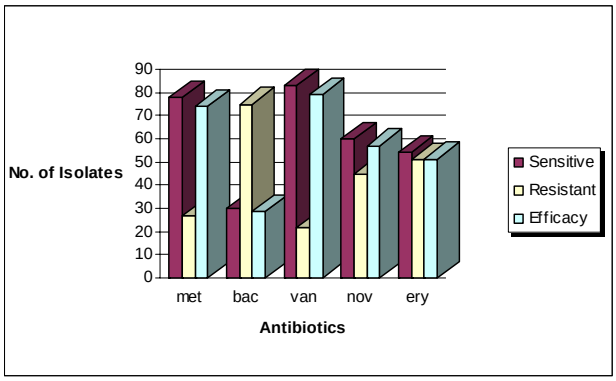

Fig 3: Total % efficacy of different antibiotics among clinical skin infection isolates.

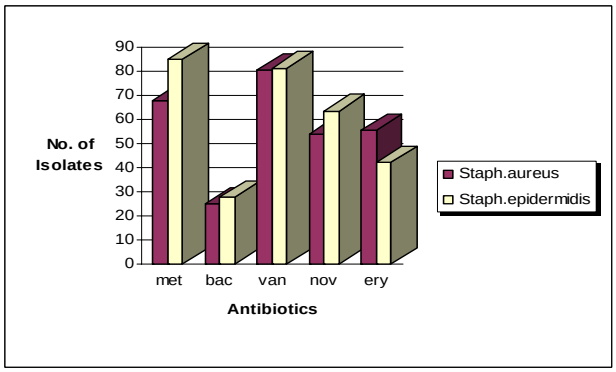

Fig 4: % Efficacy of different antibiotics against *Staphylococcus aureus* and *Staphylococcus epidermidis.*

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