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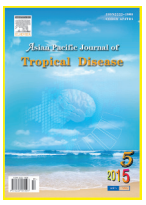

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Study of the genetic traits associated with antibiotic resistance in *Staphylococcus aureus* isolated from skin wards of Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Objective: To investigate the prevalence of *Staphylococcus aureus* (*S. aureus*) isolated from skin wards of the hospitals of Khyber Pakhtunkhwa, its resistance against various commonly and commercially available antibiotics, as well as different genetic traits of resistance and their correlations with the phenotypic visible resistance.

Methods: In the present study a simple PCR technique were used to investigate the genetic traits of resistance in *S. aureus* isolated from skin wards of two major hospitals of Khyber Pakhtunkhwa, Pakistan. A total of 100 samples were collected from both the male and female, of which 50 were from patient's site of infection and 50 from ward environment.

Results: These results demonstrated that the total prevalence of *S. aureus* both in ward as well as in patients was 48%. The *S. aureus* prevalence was the highest in female patients (50%) followed by ward environment (29%) and then male patients (21%). The antibiotic sensitivity tests revealed that the highest (91.6% isolates) sensitivity was shown to imipenem. However, the highest resistance was found to be against penicillin (100% isolates) followed by cefotaxime (75% isolates). In addition, only 29% of the isolates were found to be resistant to methicillin. PCR technique based on the previously designed primers targeting different genetic traits of resistance revealed that 13 out of the 14 isolates resistant to methicillin were positive for *mecA* gene. *blaZ* Genetic traits were found in all isolates resistant to penicillin. The multi-drug resistance traits, *vgaA* and *vgaB* each was detected only in 12.5% of *S. aureus* isolates. The phenotypic character of antibiotic resistance is highly correlated to different genetic traits of resistance.

Conclusions: Based on our findings, it is concluded that antibiotic resistance in *S. aureus* strains is increasing day by day due to self-medications and medication by non-registered medical practitioners. Therefore, for quick and fast detection, we propose next-generation sequencing be utilized to screen for antibiotic resistance.

1. Introduction

Staphylococcus aureus (*S. aureus*) is one of the most intimidating pathogens commonly found on skin and mucous membranes, *e.g.* in human nose. Almost 15%-40% of all healthy human beings are found to be carriers of this opportunistic pathogen[1]. *S. aureus* is a Gram positive cocci and a facultative anaerobe which can survive in high temperature (50 ℃), salt concentrations and drying conditions[2]. Due to its formidable ability to survive in variable environmental conditions and remarkable ability to acquire resistance against antibiotics, it is considered as one of the most threatening microorganisms[3].

Different antibiotics have been used to treat some of the serious skin and other diseases including bacteremia, boils, bullous

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impetigo, cellulitis, endocarditis, folliculitis, food poisoning, lymphadenitis, lymphangitis, osteomyelitis, paronychia, scalded skin syndrome, septic arthritis, styes and toxic shock syndrome caused by *S. aureus*[4]. *S. aureus* has the ability to become resistant to almost all the available antibiotics and bactericidal agents, either by acquiring the resistant genes from other strains or mutating its own genes. Thus through evolution, *S. aureus* has become resistant to several antibiotics, to which it was previously susceptible. Penicillin-G has been used against *S. aureus* since 1940, but soon it became resistant to this drug by acquiring *beta-lactamase* genes. Later it adapted ways to become resistant to penicillinase-resistant penicillins. Until recently this bacterium has evolved strains resistant to macrolides, lincosamides, tetracycline and gentamycin[5]. For some time methicillin were appropriate drug against *S. aureus*. However, the first methicillin resistant *S. aureus* (MRSA) was detected in 1961, which is now quite common in hospitals all over the world. After the emergence of MRSA, vancomycin were reported to be the best choice against *S. aureus*, unfortunately due to its genetic evaluation, *S. aureus* has evolved strains that have shown intermediate level of resistance against vancomycin also. Thus the first on-record vancomycin-resistant *S. aureus* was isolated in 2002 in USA[6].

S. aureus is becoming more and more prevalent in hospitals and other communities. Its extraordinary ability of becoming resistant to approximately all the available antibiotics makes *S. aureus* a formidable challenge for researchers all over the world. Therefore, the present study was conducted with the objectives to determine the prevalence of *S. aureus* isolated from skin wards of the hospitals of Khyber Pakhtunkhwa, and also its resistance against various commonly and commercially available antibiotics, *i.e.*, cefixime, cefoperazone, cefotaxime, imipenem, methicillin, penicillin-G, streptomycin and ticarcillin. The study was further focused to investigate different genetic traits of resistance and to determine their correlations with the phenotypic visible resistance.

2. Materials and methods

The present study was conducted at Microbiology Research Laboratory, Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar. A total of 100 samples were collected from skin wards of both male and female at the hospitals.

2.1. Sample collections

Commercially available sterile swabs dipped in sterile normal saline were used for the collection of samples. The samples were collected from skin ward of two local hospitals. Among these 50 samples were taken from the patient surroundings in the male and female ward while the other 50 samples were taken from patient's infection site.

2.2. Preparation of selective media

These samples were directly streaked on staphylococcus selective media. The staphylococcus selective media was prepared by dissolving the ingredients in proper proportions in distilled water according to the manufacturer's instructions. The media was autoclaved at 121 ℃ for 15 min at 15-20 psi. The media was then poured in the plates and waited till its solidification. Plates were kept in incubator for 24 h to check the sterility of the media.

2.3. Inoculation on selective media

The samples were directly streaked on the staphylococcus selective media plates and were kept for incubation of 24-48 h at 35-37 ℃.

2.4. Identification of the isolates

The isolates were identified by microscopic examinations, Gram staining and several biochemical tests including catalase, coagulase, tryptic soy agar and blood hemolysis.

2.5. Maintenance of bacterial isolates

Isolated bacterial strains were purified and preserved for further studies. For short term storage, the bacterial isolates were cultured on Muller Hinton agar slants and Petri dishes and maintained at 4 ℃. These isolates were subcultured on monthly basis for routine use.

2.6. Preparation of 0.5 McFarland turbidity standard

For making 0.5 McFarland turbidity standard, 0.5 mL of 1.175% barium chloride were dissolved in 99.5 mL of 1% sulfuric acid. This solution was stored at room temperature in dark. Using standard McFarland turbidity enables us to compare the newly prepared bacterial suspension (1.5×10^8 CFU/mL).

2.7. Determination of antibiotic sensitivity by disc diffusion method

Disc diffusion method of Kirby and Bauer was used for determination of antibiotic resistance[7]. Fresh broth culture which was incubated overnight at 35-37 ℃ was used. This culture was compared with 0.5 McFarland turbidity standard, by diluting the sample with sterile normal saline. Then the prepared broth culture was spread uniformly on the sterile Muller Hinton agar plates with the help of sterile cotton swabs. The antibiotic resistance capabilities of all the bacterial isolates were determined against various commonly used antibiotics such as cefixime, cefoperazone, cefotaxime, imipenem, methicillin, penicillin-G, streptomycin and ticarcillin[8]. Using a sterilized forceps, antibiotic discs were placed carefully on inoculated Muller Hinton agar plates. All these plates were incubated at 35-37 ℃ overnight. Next day, the zones of inhibition were measured in millimeters, and the results were classified into resistant, intermediate and susceptible according to the NCCLS guidelines (Table 1)[9].

Table 1

NCCLS guidelines for proposed discs.

Disc (Potency)	Resistant	Intermediate	Susceptible
Imipenem (10 μg)	⩽ 13 mm	14-15 mm	⩾ 16 mm
Ticarcillin (85 μg)	⩽ 14 mm	15-18 mm	⩾ 19 mm
Cefotaxime (30 μg)	⩽ 14 mm	15-22 mm	⩾ 23 mm
Cefixime (5 μg)	⩽ 15 mm	16-18 mm	⩾ 19 mm
Methicillin (5 μg)	⩽ 9 mm	10-13 mm	⩾ 14 mm
Cefoperazone (75 μg)	⩽ 15 mm	16-20 mm	⩾ 21 mm
Penicillin G (10 μg)	⩽ 20 mm	21-28 mm	⩾ 29 mm
Streptomycin (10 μg)	⩽ 11 mm	12-14 mm	⩾ 15 mm