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Isolation of Imipenem Resistant *Staphylococcus aureus* from Post-Operative Pus Sample in Oral and Maxillofacial infections

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ABSTRACT

Staphylococcus aureus is most frequently isolated pathogen causing bloodstream infections, skin and soft tissue infections and pneumonia. Recently, *Staph. aureus* have evolved resistance to both synthetic and traditional antibiotics. This study was carried out to isolate pathogenic *Staph. aureus* from post-operative pus sample, and Imipenem was identified by evaluation of resistance patterns using conventional antibiotics. A total of 66 pus samples from post-operative oral & maxillofacial surgical infections were received in the Department of Microbiology, Gurunanak Institute of Dental Science & Research, Panihati, Kolkata, over a period of one year. Imipenem is a broad spectrum antibiotic widely prescribed in hospital and it is still considered as a better choice against fatal infections. The emergence of antimicrobial resistance against effective antibiotics is a global issue. The objective of study is the surveillance of imipenem against *Staphylococcus aureus* isolated from post-operative oral & maxillofacial infections. To investigate the present status of antimicrobial resistance against imipenem, 51.5% isolates of *Staph. aureus* were collected during study period. The in-vitro antimicrobial activity of imipenem was carried out by Disc Diffusion Method (Kirby-Bauer test). 29.5% of the isolates were shown to be imipenem resistant *Staph. aureus*. It is concluded that the clinical isolates have started developing resistance against imipenem.

Keywords: *Staphylococcus aureus*, Surgical post-operative oral & maxillofacial infections, Imipenem resistance.

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INTRODUCTION

Staphylococcus aureus, Gram positive cocci, is major human pathogen causing large variety of infections worldwide and predominates in surgical wound infections with prevalence rate ranging from 4.6% - 54.4% [1-5]. *Staph. aureus* causes superficial skin infections to life-threatening diseases such as endocarditis, sepsis and soft tissue, urinary tract, respiratory tract, intestinal tract, bloodstream infections [6-7]. The species is identified on the basis of physiological or biochemical characters [8], by detection of eta and etb, staphylococcal enterotoxin genes and the Sa442 DNA fragment [9-11]. *Staph. aureus* has developed resistance to most classes of antimicrobial agents. Penicillin was the first choice of antibiotics to treat staphylococcal infection. In 1944, by destroying the penicillin by penicillinase, *Staph. aureus* become resistant [12]. More than 90% S. aureus strains are resistant to penicillin [13]. Methicillin, a semi synthetic penicillin was used to treat Penicillin Resistant *Staphylococcus aureus* but resistance finally emerge in 1962 [14-15]. MRSA is mediated by the presence of PBP-2a which is expressed by an exogenous gene, mecA [16]. High prevalence of MRSA in hospitals has been reported from many states of India [17]. MRSA isolates has reached phenomenal proportions in Indian hospitals, with some cities reporting 70% of the strains are resistant to methicillin [18].

Reduced susceptibility to imipenem has become a major problem. This study aims to determine the present trends of antimicrobial resistance against imipenem by *Staph. aureus* isolated from post-operative oral & maxillofacial infections. In-vitro disk diffusion method was used to evaluate the growth of inhibition of this pathogen, since Bauer-Kirby disk diffusion technique is a simple, reliable, and reproducible way to assess the antimicrobial susceptibilities.

METHODS

This was a prospective study conducted for 18 months (March 2011 to August 2012.).

STUDY SETTING:

The study was conducted on samples from patients and participants of Gurunanak Institute of Dental Science and Research, Panihati, Kolkata-700114, North 24 parganas, West Bengal, India.

STUDY PARTICIPANTS:

The samples were collected belonged to outdoor patients of Oral surgery & maxillofacial department in Gurunanak institute of Dental science and Research in Kolkata.

COLLECTION AND PROCESSING OF SAMPLES:

Specimens were collected for case study from post-operative oral & maxillofacial infected patients, using sterile oral cavity swabs, (under the guidance of a doctor).

A total of 66 pus samples were collected from oral suffering patients. The samples were cultured aerobically in Mannitol salt agar media (Himedia, Mumbai). The plates were incubated aerobically at 37°C for 24 hrs. Streak plate technique was used to obtain pure culture of each isolate prior to identification.

IDENTIFICATION OF ISOLATES:

The isolates were identified using colony morphology with Mannitol fermentation by colour change of the medium around each colony from red to yellow (used of Mannitol salt agar), Gram staining, Catalase, Coagulase test (slide & tube method) and DNase test as described by Cheesbrough; 2002[19].

Two hours Tryptone Soya Broth (Himedia, Mumbai) (3ml) cultures at 37°C of each isolate were adjusted to McFarland turbidity (0.5), and the disc sensitivity screening conducted as described by Cheesbrough; 2002[19]. Sensitivity testing using Kirby-Bauer disc diffusion technique [Bauer et al. (1966)] [20]. Sterile swabs were used to inoculate the test organism onto the sensitivity agar (Mueller Hinton agar media) (Himedia, Mumbai). Plate was dried for five minutes. Using sterile forceps, place disks of imipenem (10 mcg) (Himedia, Mumbai) on the plate. Plate was incubated within 15 minutes after applying the disk at 37°C for 18 hours. The diameter of the zones of growth inhibition around disk was measured to the standard values provided by CLSI this pathogen was classified as sensitive (16 mm) and resistant (13 mm) [CLSI; 2007] [21]. The result value ranges are usually regarded as pinpointing of non useful curative option akin to the resistant category for treatment purpose [22]. American Typing Collection (ATCC 25923) of *Staph. aureus* was used as a control strain in antibacterial susceptibility testing.

RESULTS

All isolates *Staph. aureus* were incubated on Mueller-Hinton agar medium with imipenem (10 mcg) disk. Organisms lying within the intermediate zones were not considered as sensitive pathogen, because they did not respond to normal therapy. Out of the 66 pus samples collected 34 (51.5%) were isolated. 29.5% of the isolates were shown to be imipenem resistant *Staph. aureus*.

DISCUSSION

The development and spread of bacterial strains that are resistant to antibacterial drugs has emerged as a global problem [23]. The appearance of antibiotic resistant bacteria over the

past decades has been regarded as an inevitable genetic response to the strong selective pressure imposed by antimicrobial chemotherapy, which plays a crucial role in the evolution of antibiotic resistant bacteria. These bacteria then pass the antibiotic resistance plasmid among other bacterial cells and species [24]. In this study 29.5% of the isolates were shown to be imipenem resistant *Staph. aureus*.

This study clearly demonstrates the development of resistance for imipenem by *Staph. aureus*. Initially, Imipenem acts as an antimicrobial through inhibiting cell wall synthesis of various Gram-positive and Gram-negative bacteria. It remains very stable in the presence of beta-lactamase (both penicillinase and cephalosporinase) produced by some bacteria, and is a strong inhibitor of beta-lactamases from some Gram-negative bacteria that are resistant to most beta-lactam antibiotics. However, after the passage of time, different factors are attributable for emergence of resistance.

In conclusion, clinically isolated *Staph. aureus* strains from pus sample are resistant to beta lactam antibiotics, aminoglycosides, macrolides, quinolones, tetracycline, chloramphenicol and vancomycin that may be due to (i) inactivation of the antibiotic due to structural modification by enzymatic action, (ii) prevention of access to target by altering the outer membrane permeability, (iii) alteration of the antibiotic target site, (iv) efflux pump which pumps out the antibiotic, (v) target enzyme bypass or over production.

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