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AMOXYCILLIN/CLAVULANIC ACID RESISTANCE IN *Staphylococcus aureus* ISOLATED FROM INFECTED ORAL CAVITY

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ABSTRACT

Amoxycillin/clavulanic acid is a broad spectrum antibiotic widely prescribed in clinical and hospital settings and it is still considered as a better choice against aerobic dental infections. The emergence of antimicrobial resistance against effective antibiotics is a global issue. The objective of study is the surveillance of amoxycillin/clavulanic acid against *Staphylococcus aureus* isolated from infected oral cavity. *Staph. aureus* is a well recognized pathogen associated with a variety of clinical syndrome. The role of *Staph. aureus* in some types of oral disease may be more important than previously recognized. To investigate the present status of antimicrobial resistance against amoxycillin/ clavulanic acid, 48.9% isolates of *Staph. aureus* were collected during study from March, 2011 to May, 2012 from different department in Gurunanak Institute of Dental Science & Research, Kolkata, West Bengal, India.The in-vitro antimicrobial activity of amoxycillin/clavulanic acid was carried out by Disc Diffusion Method (Kirby-Bauer test). 73.3% of the isolates were shown to be amoxycillin/clavulanic acid resistant *Staph. aureus.* It is concluded that the clinical isolates have started developing resistance against amoxycillin/clavulanic acid due to its irrational and inappropriate use. Continuous surveillance is crucial to monitor the antimicrobial resistance of pathogens.

KEYWORDS : *Staph. aureus*, Amoxycillin/clavulanic acid resistance, infected oral cavity

Antimicrobial resistance (AMR) is a global growing issue and several reports suggest that it is an increasing problem of phenomenal proportions, affecting both developed and developing countries (Sharma et al., 2005). AMR is considered as a natural phenomenon for the survival of micro-organism. Therefore, it is imperative to slow the rate of development of AMR to a level that maintains the usefulness of the antimicrobials (Sharma et al., 2005). Accurate determination of bacterial susceptibility to antibiotics is essential for the successful management of bacterial infections and comparative analysis of antimicrobial agents. Public health officials and clinicians monitor drug resistance through appropriate reporting of the results from susceptibility tests and this can be achieved using a number of techniques, including the disk diffusion method, the broth dilution assay, and the E tests (Bonev et al., 2008). As antibiotic resistance reduces treatment efficacy, it is a time to consider routine susceptibility testing to guide individual patient treatment and surveillance of antibiotic resistance (Nweneka et al., 2009).

Amoxicillin/clavulanic acid (INN) or co-amoxiclav (BAN) is a combination antibiotic consisting of amoxicillin trihydrate, a β-lactam antibiotic, and potassium clavulanate, a β-lactamase inhibitor. This combination results in an antibiotic with an increased spectrum of action and restored efficacy against amoxicillin-resistant bacteria that produce β-lactamase. It is a broad spectrum antibiotic, more sensitive to gram-positive bacteria, and less effective against gram-negative bacteria. However, its introduction in the treatment of a broad range of clinical conditions such as the treatment of dental infections, urinary tract infections, upper respiratory tract infections, and as a prophylaxis for neutropenic patients, as well as its use in veterinary medicine, resistant strains began to emerge. Amoxycillin/ clavulanic acid is a valuable antibiotic for the empiric treatment of dental infections; nevertheless this drug resistance should be monitored adequately. Resistance is due to one or more point mutations in the binding region of the target enzyme or to a change in the permeability of the organism.

Reduced susceptibility to amoxycillin/clavulanic acid has become a major problem. This study aims to determine the present trends of antimicrobial resistance against amoxycillin/clavulanic acid by Staph. aureus isolated from infected oral cavity. 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 (Kiehlbauch et al., 2000).

METHODS

This was a prospective study conducted for 15 months (March 2011 to May 2012.)

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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.

The samples were collected belonged to outdoor patients of various departments of Gurunanak institute of Dental science and Research in Kolkata. None of the patients who were related to the case study, were provided with antibiotics (prior to a week).

Collection And Processing of Samples

Oral cavity swabs were collected for case study from oral suffering patients, using sterile oral cavity swabs, (under the guidance of a doctor).

A total of 223 oral cavity swab 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 and tube method) and DNase test as described by Cheesbrough, 2002.

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. Sensitivity testing using Kirby-Bauer disc diffusion technique (Bauer et al. ,1966). 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 amoxycillin/clavulanic acid (20+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 (20 mm) and resistant (19 mm) (CLSI, 2007). The result value ranges are usually regarded as pinpointing of non useful curative option akin to the resistant category for treatment purpose (Schwalbe et al., 2007). 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 amoxycillin/clavulanic acid (20+10 mcg) disk. Oraganisms lying within the intermediate zones were not considered as sensitive pathogen, because they did not respond to normal therapy. Out of the 223 specimens collected 109 (48.9%) were isolated. 73.3% of the isolates were shown to be amoxycillin/clavulanic acid resistant *Staph. aureus.*

DISCUSSION

Antibiotic resistance is one of the world's most pressing public health problems. The antibiotic resistant organisms can quickly spread and so threaten communities with new strains of infectious disease that are more difficult to cure and more expensive to treat. Treatment failures may arise due to the resistance offered by pathogen against effective broad spectrum antibiotics. These treatment failures and hard to treat infections may results in high death rates (Khushal, 2004). Amoxycillin/clavulanic acid is still considered as a better choice against aerobic dental infections, in this study 73.3% of the isolates were shown to be amoxy/clav resistant *Staph. aureus.*

Throughout the 1990s, *Staph. aureus* acquired more resistance to commonly prescribed antibiotics (Dajcs et al., 2004). Moreover, when low doses of antibiotics are used against bacteria, they inhibit the growth of susceptible bacteria, leaving the smaller number of already resistant bacteria to thrive and grow. These bacteria spread their resistance traits to other previously non-resistant cells then eventually affecting other cells (Craig, 1998).

The study documents the importance of *Staphylococcus aureus* as important Gram-positive pathogen and increasing resistance in commonly used antibiotics. Although the high cost and inappropriate use of antibiotics have been documented and the long courses of prophylactic antibiotic may lead to increased resistance to

antimicrobials, increased incidence of drug reactions and increased dollar costs (Namias et al., 1998).

This study clearly demonstrates the development of resistance for amoxycillin/clavulanic by *Staph. aureus.* Initially, amoxycillin/clavulanic acid was highly effective to treat different gram positive and negative bacteria. However, after the passage of time, different factors are attributable for emergence of resistance. These mainly include; high consumption of antibiotics, irrational use, incomplete course of therapy, and self-medication by patients, leading to the emergence of resistance and even treatment failures. One major cause of self-medication is poverty. India is an under developed country, people are used to treating themselves without obtaining prescriptions from physicians. The present situation is alarming, because it is not long before amoxy/clav, an effective antibiotic would be failed to treat even simple or minor infections. Curtailed follow up of regimen also creates resistance. Generally patients stop their treatment when they feel slight improvement and the microorganisms start adapting the environment rather than get killed. Governments must initiates different educational programs, seminars, workshops in collaboration with the media to make people aware of the consequences of self-medication, especially with broad- spectrum antibiotics. In addition to this, routine antimicrobial susceptibility testing must be timely performed to determine the current status of resistance against antimicrobial agents (MIC, E test, Disk diffusion method). Otherwise therapy failures may occur which increase the cost of the therapy as well as recovery time from the underlying disease.

CONCLUSIONS

Antimicrobial resistance is a globally ever increasing problem. The emergence and spread of antimicrobial resistance are complex and driven by numerous interconnected factors. The principle causes of microbial resistance are inappropriate, irrational, high consumption, and profligate use of antibiotics. The use of antimicrobials must be restricted and monitored in order to decline the resistance. Constant surveillance and antibiotic sensitivity testing is vital for patient care and to prevent treatment failure8

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