SENSITIVITY TREND OF CLINDAMYCIN AGAINST STAPHYLOCOCCUS AUREUS & STREPTOCOCCUS PNEUMONIAE: A SERVEILLENCE STUDY

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خلاصہ

کلینیکل پیروجینز کے خلاف عام طور پر استعمال ہونے والی اینٹی بائیوٹک کلنڈامین کی موجودہ antimicrobial سپیکٹرم کا تعین کرنے کے لئےclindamycin (2µg) کی وٹرو اینٹی وٹیکیٹری کی سرگرمی کا پتہ لگانے کا طریقہ استعمال کیا گیا جو کہ کل 155 طبّی تنصیبوں کا ٹیسٹ Staphylococcus aureus (N = 110) اور Streptococcus نمونیا(N = 45)پر مشتمل ہے. مریضوں کی عمر، جنس اور انفیکشن کے ذرائع کے مطابق مختلف نمونوں کو پاکستان کے مختلف سطحی لیبارٹری لیبارٹریز اور تیسری نگہداشت کی ہسپتالوں کی لیبارٹری میں جون سے ستمبر 2016 تک جمع کرایا گیا.

Clindamycin کو *Staphylococcus aureus* کے خلاف 11.27 فیصد کے مزاحمت کے ساتھ مؤثر (82.72 فیصد) پایا گیا. تاہم، ایس ایس نیومونیا کے مقابلے میں یہ نسبتاً کم antimicrobial سپیکٹرم پیش کرتا ہے.

نتائج کے مطابق یہ نتیجہ اخذ کیا جاتا ہے کہ کلنڈامین ایسS. نیومونیا سے زیادہ مضبوطی سے ایس اوورز کی ترقی رکھتا ہے اور ایس ایوریوس کے انفیکشن کے علاج کے لئے استعمال کیا جاسکتا ہے.

Abstract

A study was made to determine the current antimicrobial activity of the commonly used antibiotic clindamycin against clinical pathogens. In-vitro antibacterial activity of the clindamycin (2µg) was examined using disk diffusion method. Total 155 clinical isolates were tested comprising of *Staphylococcus aureus* (n = 110) and *Streptococcus pneumoniae* (n = 45). Samples were differentiated on the basis of patients' age, gender and sources of infection. The specimens were collected from various pathological laboratories and tertiary care hospitals of Karachi, Pakistan from June to September 2016.

Clindamycin was found to be effective (82.72%) with a resistance of 11.27% against *Staphylococcus aureus.* However it offers comparatively less antimicrobial spectrum (78.02%) towards *S. pneumoniae.*

On the basis of findings it is concluded that clindamycin holds the growth of *S. aureus* more strongly than *S. pneumoniae* and could be used to be a treatment choice for infections of *S. aureus.*

Introduction

Antimicrobial agents are considered to be the most suitable chemicals having excellent fighting characteristics against variety of pathogens. Unfortunately, owing to the emergence of microbial resistance the effectiveness of these antimicrobials become questionable and even may lead to the therapy failures (Zehra *et al.*, 2010). Antimicrobial resistance (AMR) is a global concern and its control is a key confront for the health administration currently (Smith, 1999). It is deeming to be a greatest threat for human health that expanding the rate of morbidity, mortality and health care cost with the diminishing adequacy of such agents (Cosgrove, 2006). There are various elements contributing AMR including mainly accessibility of antimicrobials beside experts' consultation or the availability of medicines over the counter (OTC). Additionally the dispensing of the drugs by common/non professional worker having no learning about the dose regimen, contraindications, incomplete course of therapy, high consumption of anti-infective agents with unreasonable use have been further advancing the resistance. Another contributing variable for AMR is the poverty/poor economical condition and ill-literacy especially in

developing countries. Pakistan being a low-income country has been facing many challenges in health care sectors (Nweneka *et al.*, 2009, Sedas, 2007, Smith *et al.*, 2002)

Surveillance studies are routinely performed globally to determine the current efficacy profile of antimicrobial agents and to acquire latest data on resistance pattern of various microorganisms. The resistance against *S. aureus* has been reported by national and international forums. The emergence of MRSA (*methicillin resistant Staphylococcus aureus*) through gene modification is also responsible for worsening of existing infections or flushing of treatment collapse (Nikaido, 2009). Despite the introduction of novel antibacterial moieties and improvements in hygiene standards, *S. aureus* is believed to be a challenging organism that still difficult to alleviate. Clindamycin; a semi synthetic derivative of lincomycin has been extensively used to treat infections of aerobic Gram positive bacteria (S*taphylococci*), anaerobics and microaerophillic organisms. It stops the survival of attacking pathogens by blocking the protein synthesis (50S ribosomal subunit) (Smieja, 1998). Hence it is successfully employed for the management of variety of skin and soft tissue infections worldwide. It offers no issues with oral absorption however; the tissue concentration is generally higher than the plasma drug levels (Frei *et al.*, 2010).

The goal could be accomplished by accurate findings of bacterial sensitivity patterns against antibiotics together with the comparative analysis of these agents. For such evaluation different techniques are available including broth dilution, disk diffusion, e tests, automated antimicrobial susceptibility testing system and genotypic methods (Bonev *et al.*, 2008). The purpose of the present study is to evaluate the current in-vitro susceptibility of clindamycin against *Staphylococcus aureus* and *Streptococcus pneumoniae* by utilizing simple and relatively inexpensive disk diffusion technique.

Methodoloy

Clinical isolates

Collection of clinical isolates was made from various tertiary care hospitals and pathological laboratories situated in different areas of Karachi, Pakistan, from June to September 2016. These isolates were separated from throat, ear wax, blood, pus, sputum and skin samples of males, females and children.

Antimicrobial disks and Media

Cartridges of standard disks of clindamycin (2µg) was procured from the local market (Oxoid) and stored in refrigerator at (2°C to 8 °C). Muller Hinton Broth (MHB) (Merck) and Muller Hinton Agar (MHA) (Merck) were prepared according to the manufacturer's instruction.

Preparation of media plates

Media plates were prepared by pouring the freshly prepared, cooled and sterilized MHA into flat bottom petri dishes having thickness of 4mm with uniform depth.

Preparation of Inoculum

To prepare inoculum sterilized wire loop was touched slightly on the surface of isolates' colonies and then transferred into the broth containing tube (4-5mL). Incubate the tubes for 4-6 h at 37°C till the desire turbidity ($1.5x10^8$CFU/mL) was achieved having the percent transmittance of 74.3% and absorbance of 0.132 (at 600nm wave length) compared with 0.5 McFarland standard (Saeed *et al.*, 2013).

Inoculation of plates

Sterile cotton swab was dipped in inoculum suspension and then streaked in three directions by 60° rotation of plates for even distribution. Plates were then left for 5 minutes to air dry.

Placement of Antimicrobial disks

Antimicrobial disks were placed on the surface of media plates by sterile forcep and then gently pressed the disk to make a firm contact between media and disks. Plates were then placed in incubator (Lotus Co model 215) at 37°C for 18-24 hr.

Interpretation

After incubation time, zone of inhibition around the disks was measured by sliding vernier caliper and the findings were compared and categorized into sensitive (S), intermediate resistant (IR) and resistant (R) as per CLSI guidelines (CLSI, 2014).

Results

In the present study sensitivity pattern of clindamycin (2µg) was determined against total of 155 clinical isolates of *Staphylococcus aureus* (n=110) and *Streptococcus pneumonia* (n = 45). These pathogens were isolated from the laboratory samples of from children, male and female of various age groups as shown in figure 1 & 2 correspondingly. Among 110 pathogens of *Staphylococcus aureus,* 50.90% samples were collected from females, 27.27% from children and 21.81% from male. Clinical pathogens were also categorized according to their age. Highest percentage of *S. aureus* was seen in the age group of 55years and above (25.45%) and least in the 26-35years (10.90%). The *S. aureus* were obtained from cultures of skin (n= 48), pus (n=30) and blood (n=20) majorly while few from throat (n=7), sputum (n=3) and ear wax (n=2). The *S.Pneumonia* were isolated only from sputum (n=33) and blood (n=12).

Sensitivity of antimicrobial disks was observed by measuring the zone of inhibition appeared around the disk and results were analyzed as per recommendation of Clinical standard laboratory institute (CLSI) manual. The break points for sensitive, intermediate resistant and resistant pathogens to the antibiotic are presented in table 1. Among 110 pathogens of *Staphylococcus aureus* 82.72% were found to be sensitive, 6.45% showed intermediate resistance and 10.81% samples were resistant against clindamycin (2µg)

Streptococcus pneumonia was isolated chiefly from the samples of children 66.66% and least from the males 24.44%. The sensitivity of clindamycin was observed about 78.08% against *S. pneumonia* as shown in see figure 3.

Table 1: Breakpoints of Clindamycin (2µg) against Clinical isolates[13]

Clindamycin (2µg)	Sensitive (S)	Intermediate Resistant (IR)	Resistant (R)
S. aureus	≥21	15-20	≤14
S. pneumoniae	≥19	16-18	≤15

Discussion

The antimicrobial lapse is the world's most pressing public health problems. The resistant micro-organisms could spread speedily and become threaten for communities. Moreover; new strains of infectious disease are difficult to cure and consequently costly to treat. Treatment failures may arise owing to the resistance offered by pathogens even against proven effective broad spectrum antibiotics ultimately high morbidity and mortality rates (Nweneka *et al.*, 2009).

Periodic susceptibility analysis must be made for better guidance of patients' treatment and also for the surveillance of antibiotic resistance (Cars *et al.,* 2008*).* In past, various techniques have been utilized by researchers to assess the sensitivity of antimicrobials against many pathogens (Bratzer *et al.*, 2004, Sharafati-chaleshtori, *et al.*, 2010, Wild, 2002). Among different in vitro methods of detection, disk diffusion method has been used most commonly to determine resistance/ susceptibility pattern of isolates against antibiotics (Nweneka *et al.*, 2009, Sedas, 2007, Gupta *et al.*, 2001).

Staphylococci are the most common group of disease causing organism found in throat, skin, soft tissues, or facial and blood infections. The higher frequency of staphylococcal infections and diverse patterns of antimicrobial resistance have led to renew the interest towards the surveillance studies (Noskin. *et al.*, 2005, Frank, *et al.*, 2002) Clindamycin is frequently used to treat skin and bone infections due to its tolerability, cost, availability in oral form and excellent tissue penetration as well. Another specialty is its ability of being accumulates in the abscesses with no renal dose adjustments (Kasten, 1999; Brook, *et al*., 2005). Furthermore the high oral absorption makes it a good option for the treatment of both methicillin-resistant (MRSA) and susceptible staphylococcal (MSSA) infections (Fiebelkorn *et al.*, 2003).

In this study, *S. aureus* was isolated chiefly from the skin and the blood samples from different individuals. Clindamycin showed increase activity against *S.aureus* (82.72%). Reeves and co-workers, in 1991 reported the effectiveness of the clindamycin to the same organism collected from four UK centers. Clindamycin was found to be sensitive with MIC of 0.5 mg/L and with $MIC_{90}$of 0.92 mg/L, suggesting the drug is useful as a second line agent for *S. aureus* infections. Another study was conducted by Kim and associates to assess the sensitivity of clindamycin to the *S. aureus* (MSSA and MRSA). Results showed about 11.5% and 84.3% resistance for MSSA and MRSA respectively (Reeves *et al.*, 1991)

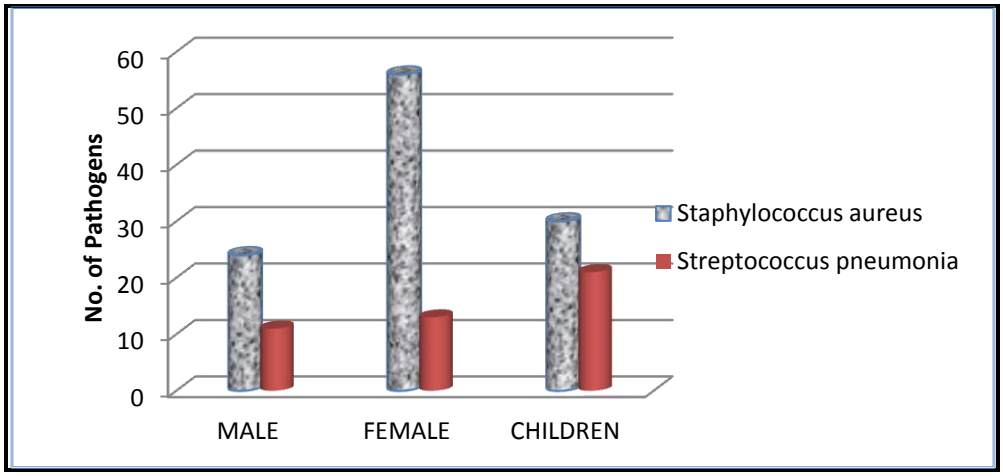

Fig.1. Organisms isolated from inhabitants-

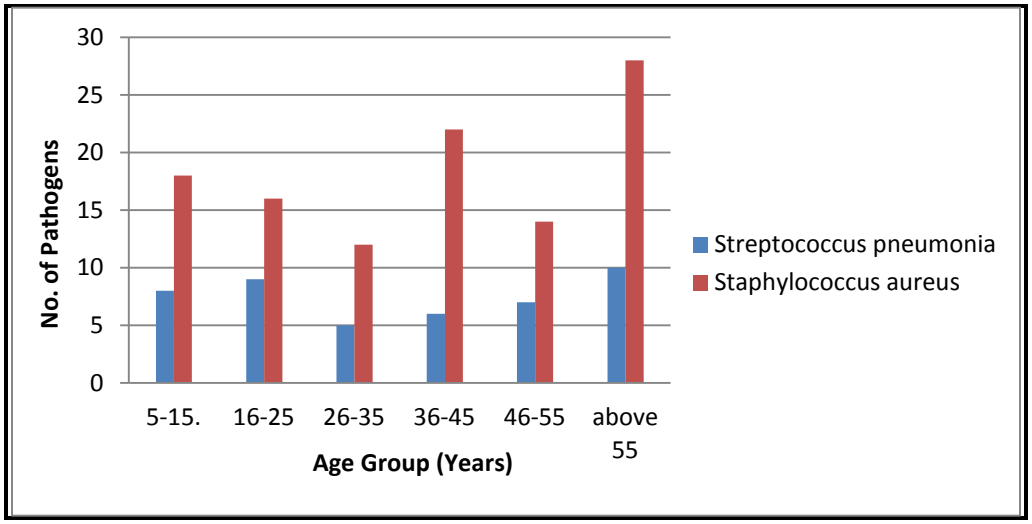

Fig.2. Distribution of clinical isolates among different age.

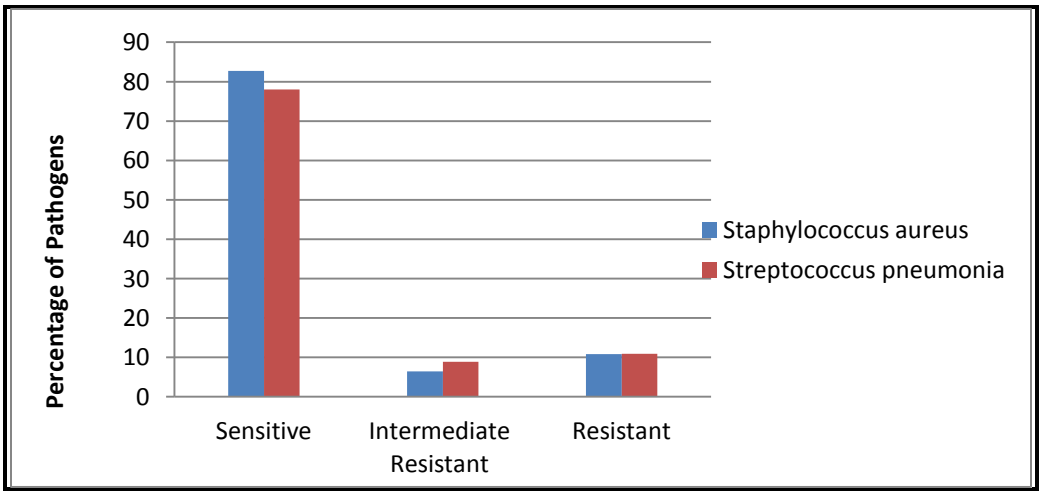

Fig.3. Susceptibility Pattern of Clindamycin (2µg) .

S*treptococcus pneumoniae* is considered to be the main source of community-acquired pneumonia (CAP) (Kelley, *et al*, 2000). Pneumococci are now found to develop resistance to th evariety of antimicrobial agents, including penicillins and macrolides (erythromycin, azithromycin and clarithromycin). It is also documented that about 15%–20% of pneumococci showed resistance to erythromycin, and even 48%–67% of strains were also non-responsive towards the penicillin (Visalli *et al.*, 1997). But still erythromycin, azithromycin, and clarithromycin are the common prescribed drugs for treating CAP. In the present investigation, sensitivity of clindamycin to the clinical strains of *S.pneumoniae* was found to be the 78.08%. This higher trend of resistance is might be due to irrational use of the drug, self medication and incomplete course of therapy. Some other contributing factors include the poor sanitation conditions with low hygienic standards.

Conclusion

In this study clindamycin was found to be effective enough when tested against clinical isolates of *Staphylococcus aureus* and *Streptococcus pneumoniae.* According to the present findings, clindamycin could be used as a first line agent to treat infections of *Staphylococcus aureus.* However; the increase in vitro resistance profile of *S. aureus* and *S. pneumoniae* is might be owing to some biochemical adaptations and irrational use of the antibiotics. Authors suggested that this study should be performed by utilizing more clinical pathogens with acknowledgment of MSSA and MRSA to observe better and entire sensitivity of the antimicrobial.

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