ORIGINAL ARTICLE

EFFICACY OF VANCOMYCIN VERSUS LINEZOLID AGAINST COAGULASE-NEGATIVE *STAPHYLOCOCCI* IN VARIOUS CLINICAL SPECIMENS

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Background: Worldwide increase in antibiotic resistance has become one of the major problems. Optimal and rationale use of antibiotic is important to prevent resistance against most of the bacteria including Coagulase-negative *Staphylococci* (CoNS), which has now been recognized as an important pathogen for nosocomial infections. This study was carried out to determine efficacy of vancomycin and linezolid against CoNS in various clinical specimens. **Methods**: A total of 2989 specimens of blood, pus and wound swab were collected from wards, casualty, intensive care units (ICU) and out-patient department (O.P.D), out of these, *Staphylococci* were isolated in 1017 specimens, of which 381 were identified as CoNS. Culture, gram stain, catalase, coagulase test and antimicrobial susceptibility pattern were done on these specimens according to clinical manual of microbiology. A total of thirteen most commonly used antibiotics were used in this study. Susceptibility testing was done by Kirby Bauer disc diffusion technique. **Results:** Antimicrobial resistance of these isolates were Amoxicillin (74.8%), Amoxicillin+clavulanate (32.8%), Ciprofloxacin (35.2%), Ofloxacin (33.6%), Ceftriaxone (30.4%), Erythromycin (58.3%), Clindamycin (16.3%), Kanamycin (52.2%) Fusidic acid (41.7%), Doxycycline (24.7%), Vancomycin (2.6%) and Linezolid (0.8%) respectively. Isolates obtained from blood were 45.9%. **Conclusion**: Vancomycin showed resistance against CoNS which is a real threat for currently applied therapy against methicilin resistant CoNS. However, linezolid efficacy is higher than vancomycin against CoNS in our study, which suggests that this drug may be considered superior to vancomycin for the treatment of infections associated with CoNS.

Keywords: Coagulase negative *Staphylococci*, Antibiotic resistance, Vancomycin, Linezolid

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INTRODUCTION

The widespread use of antimicrobial agents to treat staphylococcal infections has resulted in the emergence of resistant forms of these organisms. In the last 20 years, it has been observed that the vancomycin use has increased dramatically worldwide due to increase in prevalence of methicillin resistance in both CoNS and *Staphylococcus aureus.*[1] Previously, vancomycin was often considered the last resort and final therapeutic option for multiresistant infections. Unfortunately, decreased susceptibility of *Staphylococcus* to vancomycin has changed this approach[2] and has made it difficult to manage such cases since last few years.

The glycopeptide antibiotic vancomycin was introduced in the year 1958 for the treatment of gram-positive bacteria. Widespread use of glycopeptides recently has led to the emergence of CoNS isolates with decreased susceptibility to glycopeptides.[3,4] Resistance to vancomycin among Cons was first reported more than 20 years ago.[5] The first report of vancomycin resistance in a clinically significant isolate of CoNS was in the year 1987.[6] Since then, the resistance against vancomycin has gradually been increased and one of the studies conducted in turkey stated that vancomycin showed 9% resistance against CoNS.[7] This increasing resistance against vancomycin is a matter of serious concern for critically ill patients. Indeed, exact mechanism(s) for this resistance has not been explored yet.

Due to the above scenario, the use of newer antibiotic linezolid in recent years for the treatment of invasive infections with gram-positive organisms including *Staphylococci* is becoming popular and has shown promising results against various resistant bacteria.

Linezolid is an oxazolidinone antibiotic with bacteriostatic activity. It was discovered in 1990s and was approved by FDA in 2000 for certain gram-positive infections in adult patients including methicillin-susceptible *Staphylococcus epidermidis (*S. epidermidis) and methicillin resistant *S. epidermidis*.[8,9] In the year 2004, a study conducted in USA revealed higher efficacy of linezolid than vancomycin against Methicillin resistant *Staphylococcus aureus* nfection.[10] This finding is also supported by a meta-analysis done by An MM and colleagues in China.[11]

Coagulase-negative *Staphylococci* are reported to be the third most common causative agent of nosocomial infections and the most frequent cause

of nosocomial bloodstream infections[12,13] CoNS are a part of the normal skin microflora but can also colonize the nasal mucosa, the lower airways and invasive devices.[14,15] They produce bacteraemia mainly in patients with indwelling medical devices such as central and peripheral venous catheters, valvular prostheses, artificial heart valves, pace-makers and orthopaedic prostheses where they produce biofilm, which is the source of infection.[16]

Various international researches and studies revealed that CoNS are resistant to most of the commonly used therapeutic agents and has started showing resistance against vancomycin in clinical settings. Therefore this study was conducted to identify antibiotics susceptibility pattern of CoNS in various clinical specimens and to compare the resistance pattern of vancomycin with linezolid against CoNS in our set of population.

MATERIAL AND METHODS

This cross-sectional study was conducted at Civil Hospital Karachi from October 2012 to April 2013. A total of 2989 specimens of blood, pus and wound swab were collected from patients in wards, ICU (medical/surgical), O.P.D and casualty. *Staphylococci* were isolated in these specimens and CoNS were identified as per protocol. The specimens of pus or wound swabs were collected by disposable sterile swab stick. In suspected septicemic patients, 5ml of blood was collected by venipuncture under sterile conditions and was collected in BacTec blood culture bottle. All these samples were transferred to microbiology lab for culture and sensitivity.

On day 1, inoculation of 5ml of blood in a liquid media like BD BacTec blood culture bottle which was placed in BacTec9240 blood culture system after scanning the Bar Code, and then incubated for five days.

From day 2 onwards, when the machine beeps for positive growth, bottle was taken out after scanning as positive. It was followed by subculture on blood agar, chocolate agar and MacConkey agar. Plates were incubated at 37º C aerobically and examined for bacterial growth.

Gram stain was done from bacterial growth and gram positive cocci were processed for catalase test. Organisms which were catalase positive were processed for coagulase test. Antibiotic sensitivity was done by Kirby Baur disc diffusion technique.

On day 1, inoculation of pus and wound swab on blood agar, MacConkey agar and Robertson's cooked meat medium, followed by incubation at 37º C aerobically overnight. Gram stain was done to see pus cells and bacteria.

From Day-2 onwards, subculture from cooked meat medium on blood agar, MacConkey agar and incubation of plates at 37º C were done. These plates were examined for bacterial growth.

Gram stain was done from bacterial growth and gram positive cocci were processed for catalase test. Organisms which were catalase positive were processed for coagulase test. Antibiotic sensitivity was done by Kirby Baur disc diffusion technique.

Characteristic *Staphylococcus* colonies were identified. Gram stain, catalase and coagulase testing were done according to the manual of clinical Microbiology.

A total of thirteen antibiotics which represent the most commonly used antibiotics in the study area are used in this study. Oxacillin disc was used instead of Methicillin as a diagnostic disc to identify *Staphylococcus* as Methicillin resistant in coagulase negative *Staphylococci* (MRCoNS) and Methicillin sensitive in coagulase negative *Staphylococci* (MSCoNS).

Susceptibility testing was done by Kirby Bauer Disc diffusion technique. Zones of inhibition were measured around disk and were interpreted according to National Committee for Clinical Laboratory Standards Guidelines (NCCLS).[17]

All confirmed *Staphylococcus* isolates are screened for oxacillin (5µg) Amoxicillin (25µg), Amoxicillin+clavulanate (30µg), Doxycycline (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), Ofloxacin (5µg), Clindamycin (2µg), Vancomycin (30µg), Linezolid (30µg), Kanamycin (30µg), Erythromycin (15µg), Fusidic acid (10µg).

Data was entered on SPSS-20. Chi square test was applied to find association between either vancomycin or linezolid resistance with age groups, diagnosis, source of specimen and different departments. P value less than 0.05 was considered statistically significant.

RESULTS

Age range of patients was from 18–80 years and was divided into four groups, Young (<25 years), Young adults (25–45 years), Middle age (45–65 years) and old age (>65 years). Patients were also divided into five groups on the basis of their clinical diagnosis that are as follows; a) suffering from cutaneous abscess b) post-operative complications c) infections secondary to cancer lesions d) respiratory tract infections and e) meningitis. A total of 2989 specimens were collected and out of these, *Staphylococci* were isolated in 1017 specimens, of which 381 were identified as coagulase negative *Staphylococcus*. Majority of the isolates were obtained from blood 175 (45.9%), followed by pus 140 (36.7%) and wound swab 66 (17.3%). Antibiotic resistance pattern of CoNS showed that these isolates were resistant against most of the antibiotics as shown in figure-1, but vancomycin (2.6%) showed

comparatively higher resistance than linezolid (0.8%).Vancomycin resistance in patients who were suffering from Cutaneous abscess, post-operative complications, infections secondary to cancer lesions, respiratory tract infections and meningitis showed non-significant association and was found as 1.6%, 1.5%, 4.5%, 4.0% and 0.0% respectively , while for linezolid, pattern of resistance was found as 0.0%, 0.0%, 0.0%, 0.8% and 9.5% respectively (*p*-Value <0.05)(Table-1). Pattern of vancomycin resistance was found as 0.0%, 1.3%, 4.8% and 3.3%, while for linezolid, it was found as 0.0%, 1.3%, 0.8% and 0.0% in patients who were divided on the basis of age as young, young adults, middle age and old (*p*-Value >0.05) (table-2). In patients who were admitted in casualty, O.P.D, Medicine, Surgery, Orthopedics, I.C.U and S.I.C.U. Pattern of vancomycin resistance was 7.1%, 4.0%, 1.0%, 2.5%, 0.0%, 0.0% and 0.0%, while for linezolid it was found as 0.0%, 0.0%, 2.0%, 0.0%, 0.0%, 3.1% and 0.0% respectively (*p*-Value >0.05) (Table-3).

Table-1: Pattern of drug resistance against CoNS in patients suffering from different infections

Diagnosis	# Vancomycin Resistant n (%)	*Linezolid Resistant n (%)	Total n (%)
Cutaneous Abscess	2 (1.6)	0 (0.0)	126 ((33.1)
Post-operative complications	1 (1.5)	0 (0.0)	66 (17.3)
Infections sec.to Cancer lesions	2 (4.5)	0 (0.0)	44 (11.5)
Respiratory tract infection	5 (4.0)	1 (0.8)	124 (32.5)
Meningitis	0 (0.0)	2 (9.5)	21 (5.5)
Total	10 (2.6)	3 (0.8)	381 (100.0)

#=*p*-value 0.697, *=*p*-value 0.009

Table-2: Drug resistance against CoNS in patients of different age groups

Age	#Vancomycin Resistant n (%)	*Linezolid Resistant n (%)	Total n (%)
<25 yrs	0 (0.0%)	0 (0.0%)	47 (12.3%)
25–45 yrs	2 (1.3%)	2 (1.3%)	149 (39.1%)
45–65 yrs	6 (4.8%)	1 (0.8%)	125 (32.8%)
>65yrs	2 (3.3%)	0 (0.0%)	60 (15.7%)
Total	10 (2.6%)	3 (0.8%)	381 (100%)

#=***p***-value 0.199, *=***p***-value 0.697

Table-3: Drug resistance against CoNS in patients of different departments

Departments	#Vancomycin Resistant n (%)	*Linezolid Resistant n (%)	Total n (%)
Casualty	5 (7.1)	0 (0.0)	70 (18.4)
O.P.D	2 (4.0)	0 (0.0)	50 (13.1)
Medicine	1 (1.0)	2 (2.0)	100 (26.2)
Surgery	2 (2.5)	0 (0.0)	80 (21)
Orthopaedics	0 (0.0)	0 (0.0)	20 (5.2)
I.C.U	0 (0.0)	1 (3.1)	32 (8.4)
S.I.C.U	0 (0.0)	0 (0.0)	29 (7.6)
Total	10 (2.6)	3 (0.8)	381 (100.0)

#=***p***-value 0.164, *=***p***-value 0.412

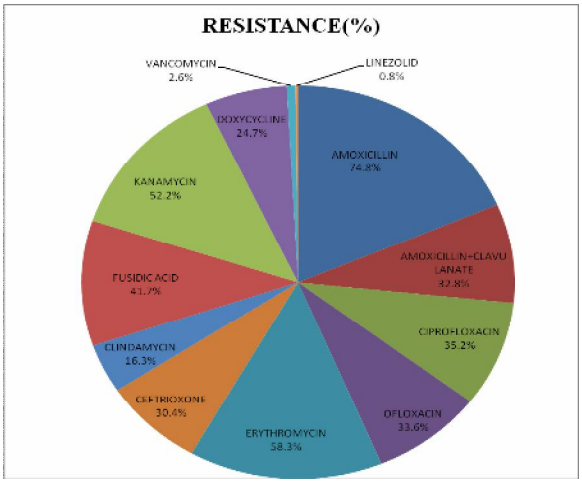

Figure-1: Antibiotic resistance pattern of various cons

DISCUSSION

Antibiotic resistant CoNS have emerged as an important agent for nosocomial infections in hospital settings.[18,19] In present study majority of the CoNS were obtained from specimens of blood as compared to pus and wound swab which is nearly similar to the study done in Iran[20] and Tunisia[21] in which number of CoNS isolates in blood were 25.4% and 29% respectively. However results were different in comparison to studies done in Pakistan[22] and India[23,24] which showed higher number of CoNS isolates in pus. This high incidence of CoNS in pus raises the impression that CoNS are ranked first among other organisms causing infected wounds and abscess, and this could be due to irrational use of antibiotics, inadequate sterilization of instruments during operation or improper care of the wounds. In our study CoNS showed 2.6% resistance against vancomycin, which is different from the studies done in Oman[25] and India[26] in which CoNS showed 100% susceptibility to vancomycin. However results are nearly similar to the study conducted in India[27] and Nigeria[28] in which CoNS showed 12.5% and 11.4 % resistance against vancomycin. Present study showed that linezolid has 0.8% resistance, which is nearly similar to the study done in Spain[29] in which none of the CoNS were resistant to linezolid. In present study, patients were divided into five groups according to different types of infections and our data showed highest vancomycin resistance (4.5%) in a group who suffered from infections secondary to cancer lesions, while patients in the same group showed 100% susceptibility against linezolid. In other groups also, linezolid is almost 100% susceptible in comparison with vancomycin except for two cases of meningitis. These results are indicating higher efficacy of linezolid as compared to vancomycin in patients suffering from different infections caused by CoNS. It has been observed that patients who were admitted in different

departments, highest resistance of 7.1% against vancomycin were present in patients visiting casualty, while the same department showed 100% susceptibility to linezolid. Patients were further divided according to different age groups and those having age of 45–65 yrs showed maximum resistance of 4.8% against vancomycin and only 0.8% resistance against linezolid. In other age groups, linezolid efficacy was also higher than vancomycin.

CONCLUSION

Emerging resistance of vancomycin against CoNS in clinical settings has become a serious threat since last many years, as this drug was considered a last resort for various multi drug resistant bacteria. Fortunately linezolid, a comparatively newer antibiotic, is showing higher efficacy against CoNS, as evidenced by international literature and also supported by the present study. Therefore, irrational use of vancomycin and linezolid should be avoided in order to prevent resistance against both the drugs in hospital settings.

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