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Assessment of antibiotic susceptibility pattern in clinical isolates of staphylococcus aureus

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Abstract
S. aureus produces various infections ranging from minor skin infection to life-threatening infections. Methicillin resistant staphylococcus aureus (MRSA) strains has emerged as one of the commonest nosocomial pathogens. Infection with MRSA strains is likely to be more severe, requires longer hospitalisation and is associated with considerable increase in morbidity, mortality and health care costs. MRSA infection is a serious concern, because of its resistance to commonly used antibiotics. Many of the MRSA isolates are becoming multidrug resistant and are showing susceptibility only to glycopeptides such as vancomycin. At present, they are showing low level resistance even to vancomycin. Hence present study was planned to evaluate the Antibiotic susceptibility pattern among clinical isolates of staphylococcus aureus with special reference to vancomycin.
The present study was planned in the Department of Microbiology, Nalanda Medical College and Hospital, Patna, Bihar from July 2018 to Jan 2019. Total of 100 non-repetitive clinical isolates of Staphylococcus aureus were isolated from various clinical specimens received in microbiology. These isolates were identified by conventional phenotypic methods such as colony morphology, Gram's stain, catalase test, slide and tube coagulase test, growth on mannitol salt agar, DNase production, gelatinase production and phosphatase production.
The results of the present study highlights the alarming development of resistance to almost all the drugs with a very few exception of drugs used in this study. Non-empirical /inappropriate antibiotic use contributes to the emergence of antimicrobial resistance in bacteria both Gram negative and Gram positive organisms. Vancomycin still remains the mainstay of treatment for serious enterococcal infections, if the strain is found susceptible. The data on local patterns of susceptibility of vancomycin resistant enterococci to newer antimicrobial agents can help in guiding the treatment of these pathogens.

Keywords: antibiotic susceptibility, staphylococcus aureus, vancomycin, etc

Introduction
Antimicrobial susceptibility tests are used to determine which specific antibiotics a particular bacteria or fungus is sensitive to. Most often, this testing complements a Gram stain and culture, the results of which are obtained much sooner. Antimicrobial susceptibility tests can guide the physician in drug choice and dosage for difficult-to-treat infections [1].
Results are commonly reported as the minimal inhibitory concentration (MIC), which is the lowest concentration of drug that inhibits the growth of the organism. Reports typically contain a quantitative result in µg/mL and a qualitative interpretation. The interpretation usually categorizes each result as susceptible (S), intermediate (I), resistant (R), sensitive-dose dependent (SD), or no interpretation (NI).
Staphylococcus aureus is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen [2]. Although S. aureus usually acts as a commensal of the human microbiota it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of S. aureus such as methicillin-resistant S. aureus (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for S. aureus has been approved.
Today, S. aureus has become resistant to many commonly used antibiotics. In the UK, only 2% of all S. aureus isolates are sensitive to penicillin, with a similar picture in the rest of the world. The β-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin, and flucloxacillin) were developed to treat penicillin-resistant S. aureus, and are still used as first-line treatment. Methicillin was the first antibiotic in this class to be used (it was introduced in 1959), but, only two years later, the first case of methicillin-resistant Staphylococcus aureus (MRSA) was reported in England [3].
Despite this, MRSA generally remained an uncommon finding, even in hospital settings, until the 1990s, when the MRSA prevalence in hospitals exploded, and it is now endemic [4].
MRSA infections in both the hospital and community

setting are commonly treated with non-β-lactam antibiotics, such as clindamycin (a lincosamine) and co-trimoxazole (also commonly known as trimethoprim/sulfamethoxazole). Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram-positive antibiotics, such as linezolid, because of its availability as an oral drug. First-line treatment for serious invasive infections due to MRSA is currently glycopeptide antibiotics (vancomycin and teicoplanin). A number of problems with these antibiotics occur, such as the need for intravenous administration (no oral preparation is available), toxicity, and the need to monitor drug levels regularly by blood tests. Also, glycopeptide antibiotics do not penetrate very well into infected tissues (this is a particular concern with infections of the brain and meninges and in endocarditis). Glycopeptides must not be used to treat methicillin-sensitive S. aureus (MSSA), as outcomes are inferior [5].

Because of the high level of resistance to penicillins and because of the potential for MRSA to develop resistance to vancomycin, the U.S. Centers for Disease Control and Prevention has published guidelines for the appropriate use of vancomycin. In situations where the incidence of MRSA infections is known to be high, the attending physician may choose to use a glycopeptide antibiotic until the identity of the infecting organism is known. After the infection is confirmed to be due to a methicillin-susceptible strain of S. aureus, treatment can be changed to flucloxacillin or even penicillin, as appropriate.

Vancomycin-resistant S. aureus (VRSA) is a strain of S. aureus that has become resistant to the glycopeptides. The first case of vancomycin-intermediate S. aureus (VISA) was reported in Japan in 1996 [98]; but the first case of S. aureus truly resistant to glycopeptide antibiotics was only reported in 2002 [99]. Three cases of VRSA infection had been reported in the United States as of 2005 [6].

High-level vancomycin resistance in S. aureus has been rarely reported [7]. *In vitro* and *in vivo* experiments reported in 1992 demonstrated that vancomycin resistance genes from Enterococcus faecalis could be transferred by gene transfer to S. aureus, conferring high-level vancomycin resistance to S. aureus [8]. Until 2002 such a genetic transfer was not reported for wild S. aureus strains. In 2002, a VRSA strain (/ˈvɜːrsə or /viːɑːrɛseɪ/) /was isolated from a patient in Michigan [9]. The isolate contained the mecA gene for methicillin resistance. Vancomycin MICs of the VRSA isolate were consistent with the VanA phenotype of Enterococcus species, and the presence of the vanA gene was confirmed by polymerase chain reaction. The DNA sequence of the VRSA vanA gene was identical to that of a vancomycin-resistant strain of Enterococcus faecalis recovered from the same catheter tip. The vanA gene was later found to be encoded within a transposon located on a plasmid carried by the VRSA isolate. This transposon, Tn1546, confers vanA-type vancomycin resistance in enterococci [10].

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Methodology

The present study was planned in the Department of Microbiology, Nalanda Medical College and Hospital, Patna, Bihar from July 2018 to Jan 2019. Total of 100 non-repetitive clinical isolates of Staphylococcus aureus were isolated from various clinical specimens received in microbiology. These isolates were identified by conventional phenotypic methods such as colony morphology, Gram's stain, catalase test, slide and tube coagulase test, growth on mannitol salt agar, DNase production, gelatinase production and phosphatase production.

The specimens were cultured on blood agar and MacConkey agar plates and incubated aerobically at 37oc for 48 hours. Identification of the isolates was carried out by standard laboratory operating procedures (Gram staining, catalase test, slide and tube Coagulase test and growth on Mannitol salt agar).8 Antibiotic sensitivity testing of all the strains was performed by modified Kirby-Bauer disc diffusion method as per CLSI guidelines

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Following was the inclusion and exclusion criteria for the present study.

Results & Discussion

The emergence of resistance to the most common anti-enterococcal antibiotics which include the β-lactam antibiotics like ampicillin, aminoglycosides and most importantly glycopeptides like vancomycin besides being inherently resistant to many others like cephalosporins and clindamycin has made the treatment of these infections a real challenge for clinicians [11]. With the increase in emergence of resistance in staphylococcus aureus to vancomycin, treatment of these infections has become difficult especially in serious infections [12]. Since options for the treatment of patients with vancomycin resistant enterococci (VRE) are very limited, this study was aimed to assess the potential usefulness of compounds, which have come into recent use. Newer antibiotics such as linezolid, daptomycin and tigecycline have shown good *in vitro* activity against VRE [13].

Table 1: Number of Isolates from Different Samples

Sample	Number of S. aureus isolates
Pus	64
Blood	10
Sputum	5
Urine	5
Nasal swab	4
Suction tip	2
Ear swab	2
Endotracheal tube	2
Pleural fluid	2
Bronchoalveolar lavage	2
Cerebrospinal fluid	2
Total	100

Table 2: Antibiotic susceptibility pattern of S. aureus isolates

Antibiotic	No. of Resistance Cases
Amoxyclav	11
Vancomycin	21
Ciprofloxacin	23
Erythromycin	14
Clindamycin	28
Co-trimoxazole	11
Teicoplanin	4
Mupirocin	3
Linezolid	1

Staphylococcus aureus are recognized as opportunistic pathogens and are natural inhabitants of the oral cavity, gastrointestinal tract (GIT) and the female genital tract in both humans and animals [14]. They have emerged as important nosocomial pathogens [15]. There are two main species - Enterococcus faecalis and E. faeciumresponsible for human enterococcal infections [16]. The most frequent infections caused by these organisms include urinary tract infections, intra-abdominal and intra-pelvic abscesses. These are increasingly being isolated from bacteraemia and meningitis cases mainly from hospitalized patients [17]. The emergence of resistance to the most common anti-enterococcal antibiotics which include the β-lactam antibiotics like ampicillin, aminoglycosides and most importantly glycopeptides like vancomycin besides being inherently resistant to many others like cephalosporins and clindamycin has made the treatment of these infections a real challenge for clinicians [18]. With the increase in emergence of resistance in enterococci to vancomycin, treatment of these infections has become difficult especially in serious infections.

Protective measures for health care workers against MRSA include contact isolation of the patient, using protective gown, gloves, mask and googles and most importantly cleaning hands with alcoholic solution at glove removal and between patients. These measures are also of paramount importance to prevent the transmission of MRSA from patient-to patient [19, 20].

For many years MRSA has been considered a typical nosocomial pathogen. In recent years, its epidemiology has radically changed, now observed even more frequently in community [21, 22]. This can be explained due to misuse of antibiotics which is a major concern of interaction by health agencies.

Conclusion

The results of the present study highlights the alarming development of resistance to almost all the drugs with a very few exception of drugs used in this study. Non-empirical /inappropriate antibiotic use contributes to the emergence of antimicrobial resistance in bacteria both Gram negative and Gram positive organisms. Vancomycin still remains the mainstay of treatment for serious enterococcal infections, if the strain is found susceptible. The data on local patterns of susceptibility of vancomycin resistant staphylococcus aureus to newer antimicrobial agents can help in guiding the treatment of these pathogens.

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