<u>**Research Article**</u>

Antimicrobial Sensitivity Pattern Of *Staphylococcus Aureus* isolated From Pus From tertiary Care Hospital, Surendranagar, Gujarat And Issues Related To The Rational Selection Of Antimicrobials

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Abstract: Antimicrobial resistance is not only increasing the healthcare costs, but also the severity and death rates from certain infections that could have been avoided by prudent and rational use of the existing and newer antimicrobial agents. Prudent and rational use of antimicrobial is possible by forming local, national and global wide antibiogram. The present study is undertaken to prepare local antibiogram of *Staphylococcus aureus* (*S. aureus*) isolated from pus and to discuss general issues related to antimicrobials use. Total 216 pus samples were processed for culture sensitivity testing. Identification of bacteria was done by gram staining. Isolation of the organisms was done by inoculation of samples on agar medium. After 24 hours of inoculation, each organism was identified on the basis of morphology of colony in culture media and biochemical reactions. Antibiotic sensitivity Testing was carried out on Muller Hinton Medium by Disc Diffusion Method following Kirby Bauer method. Out of 216 pus samples were processed, 168 organisms were isolated from pus which includes *S.aureus* (60), *Klebsiella* spp. (36), *Pseudomonas* (32), *Escherichia coli* (24), *streptococci spp.* (16) and others (48). *S. aureus* is most common organism (27.8 %) isolated from pus. *S. aureus* is most sensitive to linezolid (86.7%) and gentamicin (86.7 %). An attempt has been made in this study to recognize the *S.aureus* from pus samples in patients of tertiary care hospital, Surendranagar, Gujarat; to record the antibiogram and probable drug of choice for *S.aureus.* Considering the antibiotic susceptibility testing, cost, side effects and many other factors; cloxacillin, ampicillin/sulbactam, clindamycin, chloramphenicol and gentamicin should be preferred drugs for *S. aureus* infection isolated from pus.
Keywords: Pus, *Staphylococcus aureus*, antibiotic susceptibility testing, antimicrobial resistance, rational selection of antimicrobials

INTRODUCTION

Pus is a whitish yellow or yellow substance made primarily of dead white blood cells and dead bacteria; normally found in region of bacterial infections. It is produced during inflammatory pyogenic bacterial infections[1]. Accumulation of pus in an enclosed tissue space is known as abscess. A visible collection of pus within or breath the epidermis, on the other hand, is known as a collection of pustules or pimple [2].Pus is produced from the dead and living cells which travel into the intercellular spaces around the affected cells[3]. There are different microbial species which are responsible for the pus formation in wounds, ear infections and brain abscess. These include different bacterial and fungal species. The most common pus producing bacteria are *Staphylococcus aureus (S. aureus), Klebsiella spp.*, *Pseudomonas, Escherichia coli, streptococci.S. aureus* is most common bacteria that produce pus.

S. aureus is Gram positive cocci which is a facultative anaerobe. *Staphylococcus* was first identified in 1880 in Aberdeen, Scotland, by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint. It is commonly present on skin flora and in nasal passage. It is one of the most common causes of nosocomial infections and can also cause post-operative infections [4]. *S. aureus* is coagulase-positive; having golden yellow colonies. Local purulent infections: furuncles, carbuncles, bullous impetigo, wound infections, sinusitis, otitis media, mastitis puerperalis, ostitis, postinfluenza pneumonia, sepsis. Toxin produced by *S. aureus* cause food poisoning, dermatitis exfoliativa, toxic shock syndrome.

S. aureus has often been encountered and thought to be a Nosocomial infection (hospital acquired) and community acquired infection. In 1881, Alexander Ogston recognized that it is responsible for a number of infections in man and animals especially when there is a breakage of the skin and epithelial linings. It's now known that the organism is a medical hazard. It takes advantage when the inert and active immunity is suppressed [5].

The problem with *S. aureus* became more complicated when it was found that it quickly developed resistance and was capable of producing

many antibiotic resistant strains. This is very common in hospitals where drug resistant "hospital strains' have caused *S. aureus* infection outbreaks resulting in deaths in surgical units and newborn nurseries [6]. These are the Beta-lactam resistant strains such as MRSA that cause high mortality and morbidity.

Antibiotic resistance leads to prolonged hospital stay and increased costs in terms of treatment. In addition to these, it causes life threatening infections such as in cases of pyomyositis and chronic osteomyelitis. The majority of the MRSA strains world-wide have become resistant to multiple antibiotics including beta-lactams; tetracyclines, macrolidesand more recently fluoroquinolones [7].Excessive use of penicillin antibiotics over the years has led to the development of resistant strains of bacteria that are no longer killed by other beta lactam antibiotics.

Antimicrobial agents are among the most commonly used and misused of all drugs. The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistant pathogens, fueling an ever-increasing need for new drugs. However, the pace of antimicrobial drug development has slowed dramatically, with only a handful of new agents, few of which are novel, been introduced into clinical practice each year. Reducing the inappropriate antibiotic use is thought to be the best way to control resistance [8].

The microbiology laboratory plays a central role in the decision to choose a particular antimicrobial agent over others. First, identification and isolation of the causative organism should be taken place in the microbiology laboratory. Once the microbial species causing the disease have been identified, a rational choice of the class of antibiotics likely to work in on the patient can be made[9].

The aim and objective of the present study was to find out the prevalence and antimicrobial susceptibility of *S. aureus* isolated from pus samples and to discuss issue related to rational selection of antimicrobials in Surendranagar, Gujarat area.

MATERIALS AND METHODS

In the present study, 216pus samples from were processed in Department of Microbiology from inpatient & outpatient department of C.U. Shah Medical College & Hospital Surendranagar; from period January 2008 to October 2008.

First step done was to isolate the organisms from pus samples and then to study the culture susceptibility of*Staphylococcus aureus (S. aureus)*. Identification of bacteria was done by gram staining. All pus samples were directly inoculated onto blood agar and MacConkey agar plates and incubated aerobically at 37ºC for 24 hours. The isolates were identified with standard tests used to identify *S. aureus* such as Gram stain, catalase, slide and tube and coagulase tests. After this, sample was inoculated on plates by four flame method. Inoculated culture plates were kept in the incubator at 37° C for 24 hours [10].

Antibiotic sensitivity testing (AST) was done only for pathogenic bacteria. Antibiotic sensitivity was performed by Disc Diffusion Method of Bauer et al[11]. A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates. Filter paper disks containing designated amount of the antimicrobial drugs obtained from commercial supply firms (Himedia Labs, Mumbai, India) were used. The Mueller Hinton agar plates were allowed to dry before applying antibiotic disc. Then same commercially available antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. If an antimicrobial activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter at 24 hours using a scale. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were considered as sensitive in the assessment of the data.

From this AST, antibiogram for *S. aureus*was developed and on the basis of antibiotic sensitivity, cost effectiveness and ADR profile, appropriate antibiotic for treatment of *S. aureus*, isolated from different urine samples was achieved.

RESULTS

Table 1 Number and percentage of organisms isolated from pus

Serial number	Name of organism	Number of organism (n= 168)	Percentage of total organisms
1	*Staphylococcus aureus*	**60**	**35.8 %**
2	*Klebsiella spp.*	36	21.4 %
3	*Pseudomonas spp.*	32	19.1 %
4	*Escherichia coli*	24	14.2 %
5	*Streptococci spp.*	16	09.5 %
	Total	168	100 %

Out of 216 pus samples were processed, 168 organisms were isolated from pus which includes*Staphylococcus aureus (S. aureus)* (60), *Klebsiella* (36), *Pseudomonas* (32), *Escherichia coli and* (24) *streptococci aureus (16). S. aureus* is most common isolated organism (35.8 %).

Table-2 Antibiotic sensitivity of *S. aureus* isolated from pus-

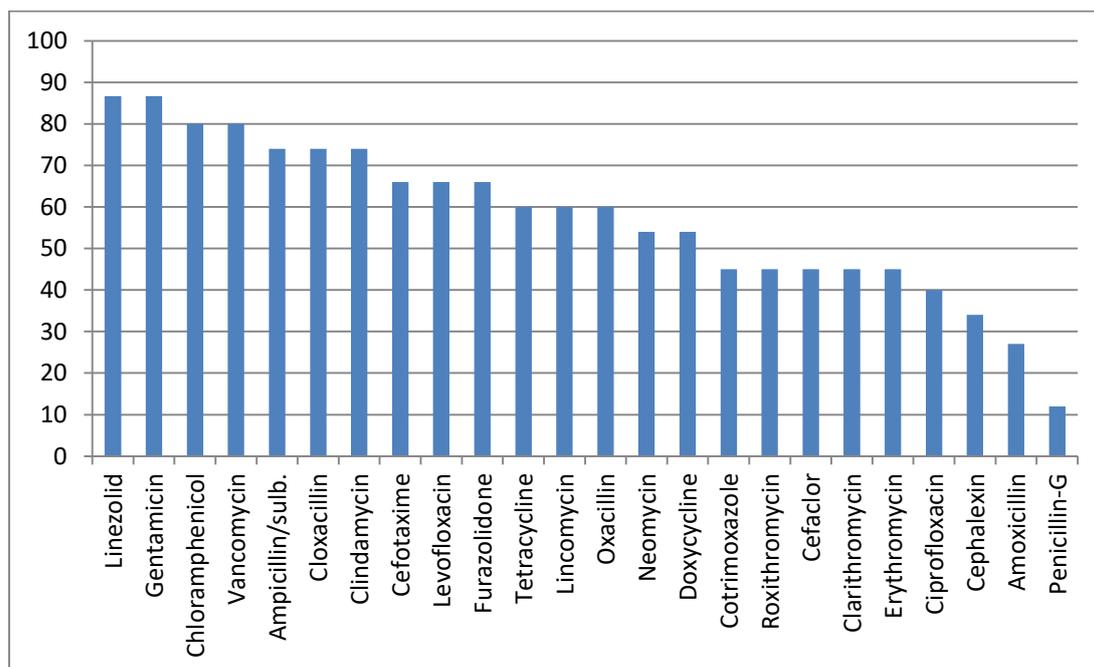| Antibiotics	Antibiotic sensitivity in %	Antibiotic Resistance in %
Linezolid	**86.7**	13.3
Gentamicin	**86.7**	13.3
Chloramphenicol	**80.3**	19.7
Vancomycin	**80.3**	19.7
Ampicillin/sulb.	**74.6**	25.4
Cloxacillin	**74.6**	25.4
Clindamycin	**74.6**	25.4
Cefotaxime	66.3	33.7
Levofloxacin	66.3	33.7
Furazolidone	66.3	33.7
Tetracycline	60.2	39.8
Lincomycin	60.2	39.8
Oxacillin	60.2	39.8
Neomycin	54.1	45.9
Doxycycline	54.1	45.9
Cotrimoxazole	45.2	54.8
Roxithromycin	45.2	54.8
Cefaclor	45.2	54.8
Clarithromycin	45.2	54.8
Erythromycin	45.2	54.8
Ciprofloxacin	40.3	59.7
Cephalexin	34.7	65.3
Amoxicillin	27.2	72.8
Penicillin-G	12.3	87.7

Figure 1-Antibiotic sensitivity of *S. aureus* isolated from pus-

As shown in Table 1 and figure 1, *S. aureus* is most sensitive to linezolid (86.7%), gentamicin (86.7%), chloramphenicol (80.3%), vancomycin (80.3 %), ampicillin/sulbactam (74.6 %), cloxacillin (74.6%), clindamycin (74.6%), cefotaxime ((66.3 %), levofloxacin ((66.3 %) and furazolidone ((66.3 %).*Staphylococcus* is least sensitive to amoxicillin and penicillin G.

Table-3-Treatment of choice for *S. aureus* isolated from pus

Sr. No.	Name of drug	Sensi.	Route of Adm.	Price in Rs Per 10 tab /per vial	Total duration of treatment	Total cost For treatment In Rs	ADR/ Toxicity of drug
1	Linezolid	87 %	Oral/ IV	315/6 tab	Bd for 7 days	735	Mild
2	Gentamicin	87 %	IM/IV	6/vial	Bd-tds × 7 days	98-126	Mild-Mod
3	Chlorampheni-col	80%	Oral/ IV	40	500 mg QID×7-10 days	150-160	Mild-Mod
4	Vancomycin	80%	IV	175/vial	125-500 mg QID×7 days	1225-4900	Mod-severe
5	Cloxacillin	74 %	oral	68/ 10 tabs	250-500 mg 4 times/day 7 days	190	Mild

Abbr.- IV- Intravenous, IM-Intramuscular, Adm.- Administration, Sensi.- Sensitivity, ADR- Adverse Drug Reaction

Percentage antibiotic sensitivity of above 5 drugs is between 74-87 %(as shown in table-1), taking consideration of cost, adverse drug reaction and other factors, Cloxacillin should be preferred drug of choice. Alternatives aregentamicin, chloramphenicol, gentamicin and linezolid. Vancomycin should be kept as reserved drug for multidrug resistant bacteria.

DISCUSSION

This study provides the data about different bacterial species isolated from pus samples and their susceptibility to various antibiotics of current use. The frequency of Gram positive organisms was found to be 45.2 % whereas the frequency of Gram negative organisms was 54.8%.Among different bacterial species*Staphylococcus aureus (S. aureus)* is recognized as an important bacterial pathogen contributing towards hospital infections, globally.

In present study, *S. aureus* is recorded to be very least resistant to linezolid and gentamycin. These drugs are proposed to be an alternative and bettertreatment of *S. aureus* infection in this part of the country. Furthermore, sensitivity of *S. aureus* to gentamycin could mean that there is a possibility of sensitivity to other aminoglycosides such as streptomycin, neomycin and kanamycin. However, this is not totally certain as each of the aminoglycosides have a slightly different mechanism of resistance due to their different aminoglycoside modifying enzymes chromosomal mutation e.g. streptomycin and impermeability of membranes. Another alternative to gentamycin in treatment of *S. aureus* infections would be clindamycin.

In present study, it has been observed that *S. aureus*showed low resistance to older drugs like chloramphenicol.It indicates that routine exposure of bacteria only to newly developed antibiotics eliminated resistance against older out of use antibiotics and present bacterial strains have grown sensitive to these outdated agents.

In present study,beta-lactam antibiotics like amoxicillin and penicillin-G shown highest level of resistance. This is attributed to the presence of beta-lactamase producing *S. aureus* in hospital environment and 'selection pressure' due to the use of the beta-lactam antibiotics for the treatment, offering advantage for the selection colonization to more resistant beta-lactamase strains.Concurrent administration of a β-lactamase inhibitor such as clavulanate or sulbactam markedly expands the spectrum of activity of acid resistant penicillins like ampicillin, amoxicillin. Same apply to cephalosporins. The dose as well as the incidence of toxicity subsequently reduced if beta lactamase inhibitors are with penicillins or cephalosporins.

In present study, *S. aureus* has shown good sensitivity to glycopeptide antibiotic vancomycin.

Higher cost and predicted higher toxicity has restricted itsuse.Consequently lesser exposure of this antibiotic to bacteria has attributed to lesser resistance. In present study, *S. aureus* has also shown good sensitivity to cloxacillin and clindamycin.

In fact, the irrational and inappropriate use of antibiotics is responsible for the development of resistance of *S. aureus*infection toCiprofloxacin, cephalexin, amoxicillin and penicillin-G.There is a need to emphasize the rational use of antimicrobials.

There is also a need strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials [12]. Vancomycin and linezolid should be considered as a reserved drug for the treatment of severe nosocomial *S. aureus*infections.

In addition, regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of the resistance patterns. An effective national and state level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics and for better patient management.Resistance to antimicrobial agents is an emerging problem that has required clinicians to alter empiric therapy for such diseases as bacterial meningitis and has prompted laboratory researchers to rethink testing strategies. The rate at which resistant organisms develop is related to their exposure to antimicrobial agents. Control strategies must include guidelines for prudent use of antimicrobial agents. The CDC has developed recommendations to help clinicians use these agents wisely to prevent the development and spread of resistant organisms[13-14]. The emergence and spread of resistance can be reduced through appropriate or careful use of antimicrobial drugs and increasing awareness among the population to the hazards of inappropriate antimicrobial use through public health education campaign[15].

In vitro sensitivity is an important factor yet other factors given below should also be seriously considered in selecting the antimicrobial agents for an infection. For example cost of drugs for complete treatment, route of administration (oral, parenteral etc.), age (if the patient is neonate chloramphenicol is contraindicated) and pregnancy (tetracyclines are contraindicated). Other factors like allergic reactions to drugs like beta lactam antibiotic, kinetics of drugs and its concentration at the target site and mode and frequency of administration, bactericidal or bacteriostatic, efficacy/safety ratio, immunological status of the patient, ADR should also be considered[16].

CONCLUSION

Selection of drug of choice in any condition especially in infective diseases is not easy. We have to take into consideration the efficacy, safety, cost, pharmacokinetic, pharmacogenetics, convenience of administration and many other factors. In case of infectious diseases; we have to pay attention to microbial sensitivity and resistance pattern to various antimicrobials. The sensitivity pattern cannot be the sole criteria. Because it is done *in vitro* and it fails to take into account the immunological status of the patient and clinical condition of the patient. An attempt has been made in this study to recognize the most common bacterial agent of infection in Surendranagar area and to record the antibiogram of the bacteria in this area.

An attempt was again made to recognize the probable drug of choice based on antibiogram and some of the other factors namely the cost of treatment, mode of administration and adverse drug reactions.*S. aureus is* most sensitive cloxacillin, ampicillin/sulbactam, clindamycin, chloramphenicol and gentamicin.Considering the antibiotic susceptibility testing, cost, convenience of administration, adverse drug reactions and many other factors cloxacillin should to be preferredfollowed by ampicillin/sulbactam, clindamycin, chloramphenicol and gentamicin for *S. aureus*isolated from the patients of pus.

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