Antimicrobial susceptibility pattern of pus culture in a tertiary care hospital of Jharkhand, India

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INTRODUCTION

Pyogenic infections are characterized by local and systemic inflammation usually with pus formation.1 These may be endogenous or exogenous. A break in the skin can provide entry to the surface bacteria which thereby start multiplying locally. The body’s defence mechanism includes bringing immune cells into the area to fight against bacteria. Eventually, accumulation of these cells produces pus which is a thick whitish liquid.2 If the infection is caused by resistant bacteria morbidity and mortality will increase which leads to great economic loss encompassing use of more expensive antibiotics to treat infection as well as threat of resistance to them.3 In almost all cases, antimicrobial therapy is initiated empirically before the results of culture are available by keeping in mind that high mortality and morbidity are associated with septicemia and right choice of empiric therapy is of importance.4 The infections caused by resistant organisms are more likely to prolong the

ABSTRACT

Background: Antimicrobial resistance is developing day by day leading to increase not only in health care cost but also severity and death rate from certain infection that could have been avoided by rational use of existing and new antimicrobial agents. Present study is undertaken for this purpose to analyse the types of pathogens involved and their antibiotic sensitivity isolated from pus culture reports in a tertiary care hospital.

Methods: Observational study was conducted using pus culture and sensitivity reports collected retrospectively from the records maintained in the Department of Microbiology over a period of 5 months from August 2016 to December 2016 in tertiary care hospital.

Results: 85 percent pus samples were found culture positive of which microorganism isolated in decreasing order were Staphylococcus aureus, Pseudomonas, Klebsiella and E. coli. Staphylococcus aureus was sensitive to fixed drug combination of piperacillin with tazobactam, linezolid, ceftriaxone with sulbactum, levofloxacinil and ciprofloxacin and resistance to cefotaxime, cloxacillin and ampicillin. Pseudomonas was highly sensitive to fixed drug combination of cefoperazone with sulbactum, piperacillin with tazobactum, ceftriaxone with sulbactum and resistance to cloxacillin and cefotaxime. Klebsiella showed high sensitivity to piperacillin with tazobactum, cefoperazone with sulbactum, ceftriaxone with sulbactum and was found resistant with norfloxacin and amoxyccillin. E. coli showed high sensitivity in decreasing order with amikacin and gentamycin and resistance in increasing order with cefotaxime, cloxacillin, ampicillin and norfloxacin.

Conclusions: The sensitivity patterns were different for each isolated microorganism but high sensitivity was found with fixed antimicrobial drug combination and resistance to frequently used drugs.

Keywords: Antibiotics sensitivity testing, Drug Resistance, Pus culture
hospital stay, increase the risk of death and require treatment with more expensive antibiotics. The increasing frequency of antimicrobial resistance among pathogens causing nosocomial and community acquired infections is making numerous classes of antimicrobial agents less effective resulting in emergence of antimicrobial resistance. Use of antimicrobial agents cause a “selective pressure” on microbial population.

In the case of antibiotics resistance antibiotics causes a selective pressure by killing susceptible bacteria allowing antibiotics resistant bacteria to survive and multiply. Moreover, highly virulent strains and capacity to adapt quickly to changing environment worsens the situation and draws a matter of concern. The current spread of multi drug resistant bacteria from clinical isolates has increased the need for regular updates in the knowledge of the bacteriological review of pus culture reports so as to avoid the unguided empirical treatment which appears to differ in various environment.

For the treatment, the isolation of bacterium from pus is valuable, but there is also urgent need of antimicrobial therapy, so sample is taken and treatment is started and after pus culture result, patient is treated as redirected by in vitro antibiotic sensitivity test.

Early recognition of lesion and prompt initiation of antimicrobial therapy are essential for controlling the infection and preventing morbidity and improve the quality of life. Antibiotic susceptibility test is a prerequisite for the management of infections which can help to make better therapeutic choices. Hence this study was planned to evaluate the prevalence of microorganism in pus isolate and its sensitivity pattern at a tertiary care hospital, RIMS, Ranchi.

**METHODS**

This study was conducted at Rajendra Institute of Medical Sciences (RIMS), Ranchi. A total of 336 pus samples were collected and subjected to antibiotics sensitivity in the department of microbiology from inpatient and outpatient department of Rims from August 2016 to December 2016. First step was to isolate organism from pus sample and then to study culture susceptibility on them.

**Characteristics of bacterial isolates**

The sample were inoculated aseptically on blood agar and Mc. Conkey media and incubated overnight at 37°C. Bacterial isolates were identified by colony morphology, gram staining, catalase test, coagulase test, oxidase test, Methyl Red / Vogus Proskur (MRVP), Triple Sugar Iron Agar test, Citrate Utilization test, Urease test and sulphur indole motility test using standard procedure for bacterial identification.

**Antimicrobial susceptibility testing**

A total of 256 samples positive for culture, were screened for antimicrobial susceptibility testing by Kirby Bauer Disc Diffusion method on Muller Hilton agar interpreted as per CLSI guidelines. The plates were incubated at 37°C for 24 hours. Antimicrobial activity was indicated by Inhibition Zone (IZ). The diameter was measured in mm using a calibrated scale.

From antibiotics susceptibility, antibiogram for different isolates is prepared and results are interpreted based on which probable drug of choice is selected.

**RESULTS**

In the present study, 336 pus samples were collected of which 286 showed positive culture and 50 were sterile culture.

*Staphylococcus aureus* was the most frequent organism isolated from pus culture reports (n= 136, 47.55%) followed by *Pseudomonas* (n=101, 35.31%), *Klebsiella* (n=35, 12.23%) and *E. coli* (n=14, 4.89 %). The frequency distribution of antibiotics screened is shown in Figure 1.

![Figure 1: Organism isolated (in percentage).](image)

**Pattern of antibiotic sensitivity and resistance for Staphylococcus aureus**

Most common organism found in this study was *S. aureus* which showed high sensitivity to parenterally given drugs like piperacillin with tazobactam (90.90%), linezolid (79.91%) followed by ceftriaxone with sulbactum (71.32%). Among oral drugs quinolones namely levofloxacin (62.17%) and ciprofloxacin (41.20%) showed better control over *Staphylococcus aureus*. The organism was highly resistant to cefotaxime (97.79%) followed by cloxacillin (97.56%) and ampicillin (66.67%) as depicted in Figure 2.
Figure 2: Drug sensitivity pattern in *Staphylococcus aureus* (in percentage).

Figure 3: Drug susceptibility pattern in *Pseudomonas* (in percentage).
Figure 4: Drug sensitivity pattern in *Klebsiella pneumonia* (in percentage).

Figure 5: Pattern of antibiotic sensitivity and resistance for *E. coli* (in percentage).
**Pattern of antibiotic sensitivity and resistance for Pseudomonas**

The 2nd most common isolate was *Pseudomonas*. It was highly sensitive to cefoperazone with sulbactum (73%) and piperacillin with tazobactum (68%) followed by ceftriaxone with sulbactum (63%). The study revealed high resistance to cloxacillin and cefotaxime as depicted in Figure 3.

**Pattern of antibiotic sensitivity and resistance for Klebsiella**

*Klebsiella* showed high sensitivity to piperacillin with tazobactum (91.42%) followed by cefoperazone with sulbactum (80%) and ceftriaxone with sulbactum (75%). It showed no resistance to amikacin, piperacillin with tazobactum, ceftriaxone with sulbactum, klebsiella was found to be resistant to norfl Roxacin (66.67%), amoxy cillin (75%). Response to cloxacillin and cefotaxime was both equivocal as few case was high sensitive and few case resistant to the above-mentioned drugs as depicted in Figure 4.

**Pattern of antibiotic sensitivity and resistance in E. coli**

*E. coli* showed high sensitivity towards aminoglycosides namely amikacin (91%) and gentamycin (87%). *E. coli* is found resistant to, 3rd generation cephalosporin like cefotaxime (92.08%), cloxacillin (91%), ampicillin (59%), norfl Roxacin (42.85%) as depicted in Figure 5.

**DISCUSSION**

*Staphylococcus aureus* is the most common human bacterial pathogen and is an important cause of skin and soft tissue infections, endovascular infections, pneumonia, tonsillitis, pharyngitis, septic arthritis, endocarditis, enterocolitis, osteomyelitis, meningitis, toxic shock syndrome, sepsis, etc. Due to inappropriate use of antibiotics, the resistance in these strains is increasing worldwide.

Methicillin resistance was documented in 52 cases (38.23%) of Staph. aureus isolates. Methicillin resistance was verified by the CLSI (formerly NCCLS) Oxacillin screening test (NCCLS, 2000) as shown in Figure 3. MRSA does not appear to be more virulent than methicillin-sensitive *Staphylococcus aureus* (61.77%), but certainly poses a greater treatment challenge. Similar were the findings of Mohanty et al and Singh et al for methicillin resistance i.e. 38.56% and 45% respectively. The prevalence varies considerably from one region to another and among hospitals in the same city.

The isolates were found to be sensitive towards vancomycin (79.41%) and linezolid (91.17%). With this susceptibility pattern resistance towards vancomycin is found to be 19.11 %. The mechanism of vancomycin resistance has been extensively studied with the first clinical vancomycin resistance *Staphylococcus aureus* VRSA strain Mu50. Biochemical and transmission electron microscopy (TEM) examination of the Mu cell suggested that it produced increased amount of peptidoglycan.

Among the antibiotics studied staph was found highly resistant to 3rd generation cephalosporin like cefotaxime (97.79%), cloxacillin (97.56%), ampicillin (66.67%). This is in accordance with earlier the work done by Befikadu et al that have reported high resistance for penicillin G and ampicillin. This observation can be attributed in part to earlier exposure of the isolates to these drugs which may have enhanced resistant development.

The continuous genetic variation could also have contributed to the increased resistance. The increasing in the penicillin resistance isolates among *Staphylococci* strains can be explained in most cases to the production of β-lactamase enzyme that destroyed the β-lactam ring and inactivated the penicillin antibiotic and this enzyme was encoded by plasmid that easy to transfer among strains. However, penicillin congeners with beta lactamase inhibitors is showing high sensitivity towards isolates namely piperacillin with tazobactum, ceftriaxone with sulbactum and cefoperazone with sulbactum showing 99.99%, 96.32 and 76.53% respectively.

Although the quinolones are reasonably active against staphylococci in vitro, the frequency of staphylococcal resistance to these agents has increased progressively, especially among methicillin-resistant isolates. In our study quinolones is showing sensitivity ranging from 77.19% for levofloxacin to 71.32 % for ciprofloxacin. Resistance to the quinolones is most commonly chromosomal and results from mutations of the topo isomerase IV or DNA gyrase genes, although multidrug efflux pumps may also contribute. Among aminoglycosides amikacin was showing high sensitivity than gentamicin, 84.84%and 77.11% respectively.

There are several different mechanisms that enable bacteria to survive and grow in the presence of antimicrobial agents. The most common mechanism involves the production of an enzyme that breaks down the antibiotic, rendering it inactive. Bacteria utilize this mechanism to overcome the antibacterial activity of beta-lactams, aminoglycosides, and chloramphenicol.

In the case of the penicillins, macrolides and quinolones, the binding sites on the bacterial wall are modified so that the drug can no longer cross the bacterial cell wall and exert its bactericidal/bacteriostatic activity. It has been demonstrated that changes in membrane permeability result in the decreased uptake of aminoglycosides, beta-lactams and quinolones into the bacterium. In other instances, the antibiotic is able to enter the bacterium, but the development of an efflux mechanism enables the agent to be removed before it can be effective. This is the
mechanism that bacteria employ against the tetracyclines and the quinolones especially norfloxacin. Activation of efflux pumps in the bacterial cell wall can reduce the susceptibility of some bacteria to other classes of antibiotics, such as macrolides, but does not result in high-level resistance.\(^{17,18}\)

*Pseudomonas aeruginosa* is a ubiquitous and versatile human opportunistic pathogen that has implications on morbidity, mortality and healthcare costs both in hospitals and in the community.\(^{19}\)

Cytotoxic chemotherapy, mechanical ventilation, and broad-spectrum antibiotic therapy probably paved the way for increasing numbers of patients colonized and infected by this organism. This situation has also been compounded by the lack of development of new classes of antipseudomonal drugs for nearly two decades.\(^{20}\) In this study pseudomonas was showing high resistance to cefotaxime (91.09%) and ampicillin (59.41%) but combination drugs was showing high sensitivity like cefoperazone with sulbactum (73%), pipercillin with tazobactum (68%) and ceftriaxone with sulbactum (63%) as shown in Figure 3.

*P. aeruginosa* strains in this study exhibited a high rate of resistance to the third-generation cephalosporin drug - cefotaxime (92.08%). A much higher resistance to other 3rd generation cefotaxime of 75%, 86% and 93.9% had been reported in studies done in India, Bangladesh and Nepal Lesser rate of resistance to cefotaxime (40%) had been reported in another study from Andhra Pradesh, India.\(^{21-24}\)

*P. aeruginosa* is inherently resistant to many antimicrobial agents, mainly due to the synergy between multi drug efflux system or a type1 Amp C β-lactamase and low outer membrane permeability. Concurrent administration of a β-lactamase inhibitor such as tazobactum or sulbactum markedly expands the spectrum of activity of acid resistant penicillin like ticarcillin and pipercillin.

Among aminoglycosides amikacin has high sensitivity (99.99%) followed by gentamycin (95.24%). Recent study showed resistance development towards aminoglycosides most commonly involves enzymatic inactivation of the drug molecule through chemical modification. Amikacin was designed as poor substrate for the enzymes that bring about inactivation by phosphorylation, adenylation or acetylation, but some organisms have developed enzymes that inactivate this agent as well. Amikacin seems to be a promising therapy for Pseudomonal infection. Hence its use should be restricted to severe nosocomial infection in order to avoid rapid emergence of resistant strains.

Among florquinolone ciprofloxacin showed high sensitivity (95.24%) and levofloxacin (77.22%). *P. aeruginosa* synthesis an exopolysaccharide called alginate in and around itself in response to environmental condition. This allows the organism to survive. Transcription of alginate biosynthetic gene responsible for development of biofilms are advantageous for survival and growth of bacteria so pseudomonas is tough to be killed.\(^{25}\)

*Klebsiella SP* is an opportunistic pathogen and is a causative agent of several kinds of infection in human. It is one of the major pathogens in nurseries, ICU and hospital wards in spite of many effective antibiotics.\(^{26}\)

Among penicillin congeners and Penicillin like drugs the present study showed most of the isolates to be highly susceptible to fixed drug combination with one of the drug in the combinations belonging to class of beta lactamase inhibitor, namely piperacillin with tazobactum (PT) and ceftriaxone with sulbactum each showing 100% sensitivity followed by cefoperazone with Sulbactum (97.14% sensitivity) as shown in Figure 5.

This observation can be compared to early reports by Gupta et al stating 63% and 80% of isolates to be susceptible to piperacillin with tazobactum and cefopeazone with sulbactum respectively.\(^{27}\) Most of Klebsiella obtained from culture also showed high resistance to the 1st line antibiotics that are commonly prescribed such as amoxycillin (75%), followed by ampicillin (62.87%), cefoxacin (57.14%) and 3rd gen cephalosporin like cefotaxime (57.11%).

In 1997, 25.8% of *Klebsiella pneumoniae* having Extended Spectrum β lactamase mediated resistance to third generation cephalosporin was reported from Nagpur, central regions of India resistance so developed among isolates may be due to overproduction of beta lactamase enzyme.\(^{28}\) Resistance to 3rd generation cephalosporin is also on a rise due to production of extended spectrum beta lactamase (ESBL) enzyme by *K. pneumoniae*. ESBLs are so named due to their ability to hydrolyze a wide spectrum of beta-lactam drugs.

This action occurs through the hydroxylation of the beta-lactam ring in beta-lactam drugs by nucleophilic attack.\(^{29}\) Resistance may also be due to production of metallo beta lactamases (MBLs) which can be chromosomally encoded or plasmid mediated. MBLs get their name from their mechanism of hydrolysis, in which they use divalent cations, Zn\(^{++}\) being the most common, in the nucleophilic attack of beta-lactam rings.\(^{30}\) The dose as well as incidence of toxicity subsequently reduced if beta lactamase inhibitors are used with beta lactam antibiotics.\(^{31}\)

Among the florquinolones tested, the isolates showed high susceptibility to levofloxacin (100% sensitive). Reports on ciprofloxacin are not promising for its use in *Klebsiella* infections as 52.93% were sensitive and 47.05% were resistant. Earlier reports on ciprofloxacin
also states resistance from as high as 63%, Gupta et al to 76.9% Ali et al.28,32

The isolated strain of *K. pneumoniae* also exhibited considerable susceptibility to aminoglycosides like amikacin (57% high sensitivity 43% intermediate sensitivity making a total of 100%) and gentamycin (85.7%). The finding were consistent previous study reporting 88% and 78% isolates to be sensitive to amikacin and gentamycin respectively.33 Even then reports of *Klebsiella pneumoniae* resistant to aminoglycosides were reported earlier.34 Resistance to aminoglycosides is frequently due to the acquisition of modifying enzymes that vary in their substrate ranges, such as acetyl transferases, phosphorylases and adenyllyl transferases. *Klebsiella pneumoniae* that produced 16S rRNA methylases 113 were reported in 2003.35 These enzymes in *Klebsiella pneumoniae* were found to confer extraordinarily high levels of resistance to clinically useful aminoglycosides, such as amikacin, tobramycin, and gentamicin.36 A study revealed a common large plasmid (85kb) in Klebsiella pneumoniae that confers resistance to ampicillin, kanamycin and chloramphenicol and intermediate resistance to amikacin.37

Resistance so developed among isolates may be due to overproduction of beta lactam enzyme. Resistance to 3rd generation cephalosporin is also on a rise due to production of extended spectrum beta (ESBL) lactamase enzyme by *K. pneumoniae*.

The less growth percentage may due to previous exposure of patients to used antibiotics that hindered their growth or dominance of organism’s growth in culture isolates. In developing countries, antibiotics are prescribed for 44-97% of patients in hospital, often inappropriately.38,39

### Table 1: Sensitivity and resistant pattern of *Staph. aureus, Pseudomonas, Klebsiella* and *E. coli* to different drugs used.

<table>
<thead>
<tr>
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<th>Staphlococcus aureus</th>
<th>Pseudomonas</th>
<th>Klebsiella</th>
<th>E. coli</th>
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<td></td>
<td>H.S.</td>
<td>I.S.</td>
<td>R.</td>
<td>H.S.</td>
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<tr>
<td>Cefoperazone</td>
<td>61.15</td>
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<td>With sulbactum</td>
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<tr>
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<tr>
<td>Ceftriaxone</td>
<td>71.32</td>
<td>25</td>
<td>3.68</td>
<td>63</td>
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<tr>
<td>With Sulbactum</td>
<td></td>
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<tr>
<td>Linezolid</td>
<td>79.91</td>
<td>11.26</td>
<td>8.83</td>
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<td>Amoxycillin</td>
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</table>

Hs: High sensitivity; I.S: Intermediate sensitivity; R: Resistance

**Pattern of antibiotic sensitivity and resistance for E. coli**

*E. coli* showed high sensitivity to aminoglycosides like amikacin 91% compared to another aminoglycosides gentamycin (87% sensitive). Our result was further supported by another study where the susceptibility rate of *E. coli* remained 93-100%.40 The mechanism of resistance now has been found to be production of aminoglycosides modifying enzymes.

Among the quinolones levofloxacin showed inhibition in 72 % isolates while ciprofloxacin showed inhibitory activity of 63%. The susceptibility towards norfloxacin is sensistive 57.14 % and resistance 42.85%. The antibiotics belonging to beta lactam/penicillin pathway inhibitors like ampicillin, cloxacillin proved to be least active (inhibition of 40.59% and 8.91% of the total isolates respectively) indicating the increased resistance in *E. coli* towards these drugs. In case of cephalosporins it was observed that *E. coli* developed substantial resistance towards third generation drugs (sensitivity towards cefotaxime being 7.92%).

Penicillin congeners with beta lactam inhibitors are the most active and reliable agents for the treatment of infection which are caused by ESBL producing organisms.
as their growth were highly restricted with their use (cefoperazone with sulbactum, piperacillin with tazobactum) and ceftriaxone with sulbactum each showing high sensitivity of 73%, 68% and 63% respectively.

India has one of the highest rates of Gram negative bacillary resistance in the world. This is due to over reliance on broad spectrum antibiotics, which is because of diagnostic uncertainty among physicians and non-empirical or inappropriate use of antimicrobial agents.

CONCLUSION

Organism isolated from pus culture in decreasing order were Staphylococcus aureus, Pseudomonas, Klebsiella, and E. coli. Most common organism found in this study was S. aureus which showed high sensitivity to piperacillin with tazobactam, linezolid followed by ceftriaxone with sulbactum. Among oral drugs levofloxacin and ciprofloxacin showed better control over Staphylococcus aureus. The organism was highly resistant to cefotaxime followed by cloxacillin and ampicillin. The 2nd most common isolate was Pseudomonas. It was highly sensitive to cefoperazone with sulbactum and piperacillin with tazobactam followed by ceftriaxone with sulbactum. This study revealed high resistance to cloxacillin and cefotaxime. Klebsiella showed high sensitivity to piperacillin with tazobactam followed by cefoperazone with sulbactum and ceftriaxone with sulbactum; and was found resistant with norfloxacin and amoxycillin. E. coli showed high sensitivity in decreasing order with amikacin and gentamycin and resistance in increasing order with cefotaxime, cloxacillin, ampicillin and norfloxacin.

In this study only limited numbers of drugs were studied for sensitivity pattern and culture was done for common pathogenic organisms. There is need for wide range and periodical study to know the changing sensitivity pattern of microorganisms. This study may be helpful in deciding empirical therapy of an infection considering other related factors before the actual culture and sensitivity report of a microorganism comes.

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REFERENCES

17. Roberts MC. Tetracycline resistance determinants: mechanisms of action, regulation of expression,


