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# A Study on Microbiological Profile of Ear Infections in Patients Attending a Tertiary Care Hospital

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Abstract: Ear infections occur in all age groups and are one of the most common clinical presentations in Ear, Nose, and Throat (ENT) clinics, especially in developing countries like India. Various studies have been done to determine the microbiological profile of ear infections with varying results. Aim: This study was conducted at Chengalpattu Medical College and Hospital to determine the aerobic microbiological profile of ear infections in this geographical area, with an emphasis on the antimicrobial susceptibility patterns of the bacterial organisms isolated. Materials and Methods: 150 samples were taken from both sexes of all age groups who attended the ENT OPD with symptoms of ear infection, over a period of one year, from January 2018 to December 2018 in Chengalpattu Medical College and Hospital. Gram stain, KOH wet mount, bacterial culture and sensitivity, and fungal culture were carried out. Results: Bacteria accounted for 87% and fungi for 13% of the 140 culture-positive cases. Among the bacterial isolates, Gram-negative organisms predominate (64%). The most common bacterial isolate was Pseudomonas aeruginosa (42.6%), followed by Staphylococcus aureus (31.14%). The most commonly isolated organisms showed the highest sensitivity to Ciprofloxacin and Amikacin. The most common fungal isolate was Aspergillus species (78%), followed by Candida species (22%). Conclusion: This study on the microbial profile of ear infection gives a comprehensive picture of the etiological agents and their antibiotic sensitivity pattern. This helps to ensure prompt and effective treatment, thereby reducing the risk of serious complications and also the emergence of resistant strains.

Keywords: Ear infection, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus species, Candida species

#### 1. Introduction

Ear discharge (bacterial, fungal, and viral) is one of the most common clinical presentations to ENT clinics. The major causes of CSOM are Pseudomonas aeruginosa, Staphylococcus aureus, Proteus species, Klebsiella species, Escherichia coli, and anaerobes.[1] Fungus can be a primary or superimposed infection. The most dominant fungi in ear infections include Aspergillus species and Candida species.[2]

Risk Factors [3] include trauma, foreign bodies, moisture, infection from the middle ear, and the upper respiratory tract. Clinical Features [4] include Pain and tenderness, Pustule or furuncle, Edema, swelling, erythema of the canal wall, Ear discharge, Hearing loss, and Perforation. Complications [3]: Meningitis, Brain abscess, Extradural abscess, Subdural abscess, Lateral sinus thrombophlebitis.

AOM is often self-limiting [5,6]. Tympanocentesis or myringotomy can be done for drainage to relieve pain, and the material is sent for culture.[7] CSOM is treated with antibiotics & surgery in cases with tympanic membrane perforation. The antibiotics commonly used are-[7,8] Amoxicillin, Amoxicillin-clavulanate, Ciprofloxacin/Ofloxacin, Erythromycin / Azithromycin/ Clarithromycin, Cephalexin/ Cefpodoxime, Cotrimoxazole. Parenteral

antibiotics for invasive otitis include- [9] Ceftriaxone, Piperacillin, Vancomycin.

The dense mat of fungi can be removed with a wax hook through an otoscope.[5] Up-to-date information about the common causative organism and their antimicrobial susceptibility pattern is essential for the rational use of the drugs for treatment. This study aims to determine the etiological agents causing ear discharge with emphasis on the antimicrobial susceptibility patterns of the bacterial isolates.

The prevalence in India is 7.8% as per a school survey in Tamil Nadu.

### 2. Aim and Objectives

This study aims to determine the microbiological profile of ear infections in patients attending a tertiary care hospital.

The objectives are to isolate and identify the aerobic bacterial and fungal agents causing ear infections. Study the antimicrobial sensitivity and resistance pattern of the bacterial isolates. Find the prevalence of Metallo-β-Lactamase (MBL) resistance in *Pseudomonas aeruginosa* isolates. Determine the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in *Staphylococcus aureus* 

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isolates. Find the Vancomycin susceptibility of the MRSA isolates. Know the prevalence of Inducible Clindamycin Resistance (ICR) in Staphylococcal isolates.

- a) Location: The Department of Microbiology& Department of Otorhinolaryngology, Chengalpattu Medical College and Hospital.
- b) INCLUSION CRITERIA: Patients having ear discharge, both genders, of all age groups
- c) EXCLUSION CRITERIA: History of antimicrobial therapy in the last 7 days
- d) Collection of Ear Discharge: The ear discharge was collected using sterile swabs under aseptic precautions. Three samples were taken. One swab for Direct Gram Stain and KOH mount, Second swab for bacterial culture, Third swab for fungal culture

### 1. Sample Processing

Direct Gram Stain, Potassium hydroxide (KOH) wet mount was performed, Bacterial Culture, Biochemical Reactions, and Antimicrobial Susceptibility Testing were done.

## 1.1. Test For Detection of MBL in Pseudomonas Aeruginosa

All Pseudomonas aeruginosa isolates were screened for Metallo- $\beta$ -Lactamase production by the Meropenem disc diffusion method by placing a 10 $\mu$ g Meropenem disc on a lawn culture of the test organism. The zone of inhibition was determined after 16 – 18 hours of incubation at 37°C in ambient air. The zone size was interpreted as: Sensitive  $\geq$  19mm, Intermediate 16 – 18 mm, Resistant  $\leq$  15 mm. Isolates with a zone of inhibition less than or equal to 15mm were considered as MBL producers.

## 1.2. Test For Detection of ICR in STAPHYLOCOCCUS Species:

Disc diffusion method - 'D' zone test: Isolates of Staphylococcus aureus, which were resistant Erythromycin, were screened for Inducible Clindamycin Resistance by standard disc diffusion procedure. Done using a 15µg Erythromycin disc and a 2µg Clindamycin disc, placed 15-26 mm apart edge to edge, on a lawn culture of the test organism. The zone of inhibition was determined after 16 – 18 hours of incubation at 37°C in ambient air. The zone of inhibition was interpreted as: Flattening of the zone of inhibition adjacent to the Erythromycin disc, i.e., 'D' zone is Positive for Inducible Clindamycin Resistance. Hazy growth within the zone of inhibition around the Clindamycin disc is Constitutive Clindamycin Resistance.

### 1.3. Test For Detection of MRSA:

Cefoxitin disc diffusion method: Isolates of *Staphylococcus aureus* were screened for methicillin resistance by the standard disc diffusion procedure. The test was performed by placing a  $30\mu g$  cefoxitin disc on a lawn culture of the test organism. The zone of inhibition was determined after 16-18 hours of incubation at  $37^{\circ}C$  in ambient air. The zone size was interpreted as: Susceptible  $\geq 22 \text{mm} = \text{mecA}$  negative, Resistant  $\leq 21 \text{ mm} = \text{mecA}$  positive. Isolates with a zone of

inhibition less than or equal to 21mm were considered as Methicillin-resistant *Staphylococcus aureus* (MRSA).

## 1.4. Test For Detection of Vancomycin Susceptibility in MRSA

Epsilometer test: Isolates of methicillin-resistant *Staphylococcus aureus* were tested for Vancomycin susceptibility. A lawn culture of the test organism. Vancomycin E-strip was placed on it. After 16-18 hours of incubation at  $37^{\circ}\text{C}$  in ambient air, the point of intersection of the ellipse with the MIC scale on the strip is noted. Susceptible  $\leq 2~\mu\text{g/mL}$ , Intermediate  $4-8~\mu\text{g/mL}$ , Resistant  $\geq 16~\mu\text{g/mL}$ .

### 1.5. Fungal Culture

The culture of the debris material taken out from the external auditory canal using a sterile swab was inoculated onto 2 slopes of Sabouraud Dextrose Agar, supplemented with the antibiotic Gentamicin to avoid bacterial contamination, and had its pH adjusted to 5.6. The cultures were incubated at 25°C and at 37°C for 4 to 6 weeks. The slopes were examined for growth daily for the first week and twice a week for the subsequent weeks. Failure of growth even after six weeks of incubation was considered negative for fungal growth and was discarded as sterile. Tubes showing positive cultures were examined for their macroscopic and microscopic morphological features. Macroscopic examination & Microscopic examination for fungal elements. For yeast-like fungi like Candida: Gram staining, Germ tube test, and CHROM agar tests were performed.

### 3. Results

A total of 150 samples of ear discharge were collected and processed from 80 males and 70 females from the age group 1 to 80 years. Based on clinical diagnosis, there were 7 cases of otitis externa, 13 cases of acute otitis media, 114 cases of chronic suppurative otitis media, and 16 cases of otomycosis. Out of the 150 samples, 140 showed culture positivity and 10 showed no growth (Table & Figure 5).

Among 140 culture-positive cases, 122 were bacterial isolates and 18 were fungal isolates. Out of 18 fungal isolates, 12 were Aspergillus niger, 2 Aspergillus flavus, 3 Candida tropicalis, and 1 Candida glabrata. Out of 122 bacterial isolates, 52 were Pseudomonas aeruginosa and 38 were Staphylococcus aureus. The most common organism isolated in otitis externa and acute otitis media was Staphylococcus aureus. Pseudomonas aeruginosa was the most common organism in CSOM, and Aspergillus niger in otomycosis. Antimicrobial susceptibility testing was done for the bacterial isolates by the Kirby-Bauer disc diffusion method. No MBL was detected in Pseudomonas. 4 MRSA were detected, and all 4 were susceptible to Vancomycin (Table &Figure 10). Overall sensitivity among all isolates was higher for Ciprofloxacin and Amikacin.

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### 4. Discussion

Among 150 patients, the age-wise distribution of ear infection in our study shows 13.33%, (Table & Figure 1), with peak incidence in the second decade of life (18.67%). A similar analysis was made by Rajiv *et al* (24%). This may be due to lack of effective treatment, poor hygiene, poverty, and overcrowding [10,11]. Upper respiratory tract infection is also a common predisposing factor in young adults [12].

Our study shows males (53.33%) were affected more than females (46.67%) (Table & Figure 2). A similar observation was made by Madana *et al*, where males (56%) were more affected than females (44%). No data is available for sex distribution in ear infections, but male predominance can be due to their more exposed, adventurous way of life [13,10] or due to their ear cleaning habits [14].

Bilateral ear infections were seen in 3.34% of the cases. The right ear (53.33%) was affected more than the left ear (43.33%) (Table & Figure 3), which can be explained by the right-handedness of most people, thereby frequently introducing infections into the right ear. Similar observations were made in studies by Arun Ghosh *et al*, with 3.70% of bilateral cases, 66.66% right-sided sided and 29.62% left-sided ear infection.

In our study culture positivity was seen in 93.33% and negativity in 6.67% similar to a study by Nandy A. *et al*, which showed 95.54% positivity. No growth in culture may be due to prior antibiotic usage [15] or infection by strict anaerobes or viral agents [16] [11].

Among the culture-positive cases, 87.14% were aerobic bacterial isolates and 12.86% were fungi. This correlates with studies by Ravindra Singh *et al,* which say bacteria (77%) are more common than fungi (23%).

In this study Gram Negative bacilli (64%) outnumbered Gram-positive cocci (36%)(Table & Figure 6), which is similar to the study by Jyothi *et al* (GNB 65.3% and GPC 34.7%).

The most common organism is *Pseudomonas aeruginosa* (42.6%), followed by Proteus species (8.11%), *Klebsiella pneumoniae* (4.92%), *Acinetobacter baumannii* (4.92%), and *Escherichia coli* (3.29%). A similar observation was made by Raghu Kumar *et al. Pseudomonas aeruginosa* predominance can be due to its minimal nutritional requirement and its ability to grow in a moist environment.

Among the Gram-positive cocci (36%), the most common was *Staphylococcus aureus* (31.14%), followed by Coagulase Negative *Staphylococcus* (4.92%). This is in concordance with the study results of Raghu Kumar *et al-Staphylococcus aureus* 34.44% and Coagulase Negative *Staphylococcus* 3.33%.

Among the 150 cases, 4.67% were clinically diagnosed to be otitis externa, 8.67% acute otitis media, 76% chronic suppurative otitis media, and 10.66% otomycosis (Table & Figure 4).

Among the 114 CSOM cases, the most common organism was *Pseudomonas aeruginosa* (45.61%) followed by *Staphylococcus aureus* (22.81%). Other organisms isolated include Proteus species (8.77%), *Klebsiella pneumoniae* (5.26%), *Escherichia coli* (3.51%), *Acinetobacter baumannii* (5.26%) and Coagulase Negative *Staphylococcus* (5.26%) (Table & Figure 7).

All 52 *Pseudomonas aeruginosa* isolates were sensitive to Meropenem. None were Metallo-β-Lactamase producers, implying Meropenem is the most active drug against *Pseudomonas aeruginosa* in ear infections. [17, 18] Similar results were noted by Arti Agarwal *et al* and Fatima *et al*. But Jyothi *et al* and Ritu Gupta *et al* have reported 8.4% and 7.5% resistance to Meropenem, respectively.

Pseudomonas aeruginosa isolates showed 44% sensitivity to ceftazidime, 86.5% to Amikacin, 71%; Gentamycin, 85%; Ciprofloxacin, and 88.5% to Piperacillin-Tazobactam. Similar results were given by Arti Agarwal *et al*, with 80-90% sensitivity to aminoglycosides and Piperacillin-Tazobactam and 60-70% sensitivity to Cephalosporins and fluoroquinolones (Table & Figure 8). [18]

In our study, among the 52 Pseudomonas aeruginosa isolates, 3 were MDR pathogens (5.77%). The emergence of resistant isolates may be because of injudicious use of antibiotics in inappropriate doses as a result of easy accessibility [18] to these drugs by over-the-counter sales without prescription [14].

Out of the 38 Staphylococcus aureus isolates, 4 were resistant to Cefoxitin, i.e., Methicillin Resistant Staphylococcus aureus (MRSA - 10.53%). The remaining 34 isolates were Methicillin sensitive (MSSA - 89.47%). Ritu Gupta et al have noted similar results, while Madana et al and Jyothi et al have noted only 2% MRSA. >65% of Staphylococcus aureus isolates are known to be MRSA isolates (Table & Figure 9). [19]

All 4 MRSA isolates were sensitive to Vancomycin (100%), thus making Vancomycin the drug of choice for MRSA isolates [18]. 100% sensitivity to Vancomycin is also noted by Jyothi *et al*, Madana *et al*, Rakesh Kumar *et al*, and Ritu Gupta *et al*.

In our study, *Staphylococcus aureus* isolates showed 84.21% sensitivity to Ciprofloxacin and Clindamycin, 21.1% to Penicillin, 47.37% to Cotrimoxazole, and 60.53% to Erythromycin. This decreased sensitivity to Penicillin, Cotrimoxazole, and Erythromycin may be due to repeated empirical prescription of these drugs over a long period of time.

Coagulase-negative *Staphylococcus* (CoNS) is the most common organism colonizing the outer ear [20]. In our study, 4.92% of CoNS have been isolated, which is similar to the study by Yousuf *et al* (5%). Raghu Kumar *et al* and Arun Ghosh *et al* It might be a contaminant [22] or an opportunistic pathogen which can sometimes cause infection [21].

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Out of the 44 *Staphylococcus* species isolated, 19 were resistant to Erythromycin. When these 19 isolates were subjected to the 'D' zone test, no inducible Clindamycin resistance was seen. Only 8 isolates showed constitutive resistance to Clindamycin.

All isolates of the Enterobacteriaceae family, which includes *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Escherichia coli*, showed 100% sensitivity to Amikacin, Piperacillin-Tazobactam, and Meropenem. Arti Agarwal *et al* have recorded 70-80% sensitivity of Enterobacteriaceae with Cephalosporins, aminoglycosides and fluoroquinolones [18].

Acinetobacter baumannii isolates showed 100% sensitivity to Gentamycin, Ciprofloxacin, Piperacillin-Tazobactam and Meropenem. 50% resistance to ceftriaxone and 33% resistance to Cotrimoxazole were noted in our study. This is similar to a study by Raghu Kumar *et al*.

The fungi isolated in our study were Aspergillus niger (66.67%), Aspergillus flavus (11.11%), Candida tropicalis (16.67%), and Candida glabrata (5.55%). This is similar to the study by Jyothi et al, but Ravindra Singh et al and Fatima et al have reported a predominance of Candida species (75%) over Aspergillus species (25%).

The present study shows that in our setup, ear infections are commonly caused by *Pseudomonas aeruginosa*, followed by *Staphylococcus aureus*. These isolates are highly susceptible to Ciprofloxacin, Amikacin, and Piperacillin-Tazobactam, which are bactericidal agents and can be safely used in all age groups [23].

In our study, 88.46% of *Pseudomonas aeruginosa* were sensitive to Piperacillin-Tazobactam.

In our study, 86.54% of *Pseudomonas aeruginosa* were sensitive to Amikacin.

In our study, 84.62% of *Pseudomonas aeruginosa* and 84.21% of *Staphylococcus aureus* were sensitive to Ciprofloxacin.

This explains the higher sensitivity of these organisms to Amikacin, Ciprofloxacin, and Piperacillin-Tazobactam. This also explains why Metallo-β-Lactamase-producing *Pseudomonas aeruginosa* are rare in ear infections.

However, inappropriate use of these antibiotics can lead to the emergence of resistant strains. Therefore, judicious usage of these antibiotics by adhering to the antibiotic policies is essential.

### 5. Conclusion

In this study, *Pseudomonas aeruginosa* is the most common cause of ear infection, followed by *Staphylococcus aureus*. These organisms are found to be less sensitive to the commonly used drugs like Gentamycin, Amoxicillin, Erythromycin, and Cotrimoxazole. Most of the isolates were sensitive to Ciprofloxacin. This study showed that no MBLs were produced by *Pseudomonas aeruginosa*, causing ear

infection. This study also indicated the presence of MRSA strains. Cleanliness and hygiene should be followed to control their spread. Because of the frequency of drugresistant strains, antimicrobial susceptibility testing should be done for all isolates to help in the choice of drugs. CDC has developed a program for the appropriate use of antibiotics, like avoiding antibacterial drug use for trivial, viral respiratory tract infections. This should be followed to decrease the emergence of multidrug-resistant strains.

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### **Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### **Tables and Figures**

**Table 1:** Age Distribution

Age Group	No. of Cases	%
1 to 10	20	13.33
11 to 20	28	18.67
21 to 30	26	17.33
31 to 40	25	16.67
41 to 50	22	14.67
51 to 60	19	12.67
61 to 70	7	4.66
71 to 80	3	2
Total	150	100

The above table shows age wise distribution of ear infection in our study. 20 were in the age group 1-10 years (13.33%), 28 in 11-20 years (18.67%), 26 in 21-30 years (17.33%), 25 in 31-40 years (16.67%), 22 in 41-50 years (14.67%), 19 in 51-60 years (12.67%), 7 in 61-70 years (4.66%) and 3 in 71-80 years (2%).

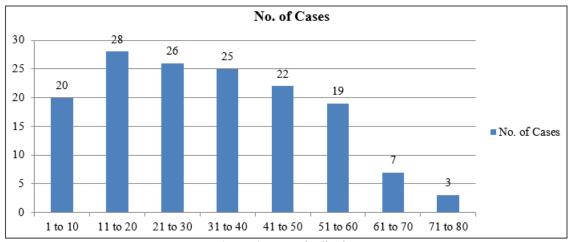


Figure 1: Age Distribution

Table 2: Gender Distribution

Sex	No. of Cases	%
Male	80	53.33
Female	70	46.67
Total	150	100

Out of 150 cases, 80 were male (53.33%) and 70 were females (46.67%). Among them 73 males and 67 females were culture positive.

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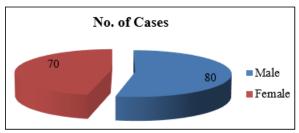


Figure 2: Gender Distribution

Table 3: Ear Involved

Ear	No. of Cases	%
Right	80	53.33
Left	65	43.33
Bilateral	5	3.34
Total	150	100

Out of 150 cases, 145 were unilateral (96.66%) and 5 were bilateral (3.34%). Right sided ear involvement was seen in 80 cases (53.33%) and left side in 65 cases (43.33%)

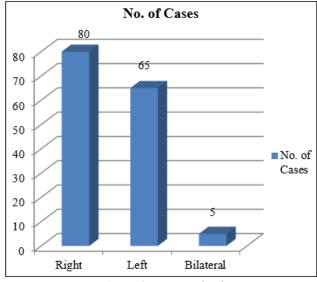


Figure 3: Ear Involved

**Table 4:** Types of Ear Infections

No. of Cases	%
7	4.67
13	8.67
114	76
16	10.66
150	100
	7 13 114 16

Out of 150 cases, a majority of 114 were CSOM cases (76%). 16 otomycosis (10.66%), 13 AOM (8.67%) and 7 OE (4.67%) cases were noted.

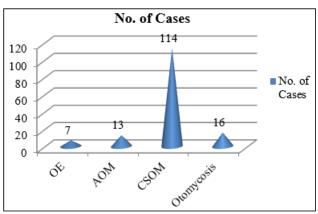


Figure 4: Type of Ear Infection

**Table 5:** Culture Results

Culture	No. of Cases	%
Positive	140	93.33
Negative	10	6.67
Total	150	100

Out of 150 culture samples, only 10 showed no growth (6.67%); culture results were positive for the remaining 140 samples (93.33%).

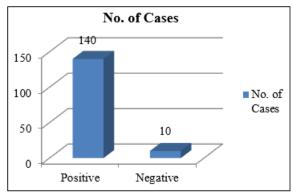


Figure 5: Culture Results

Table 6: Microbiological Profile

Isolates	No. of Cases	%
GPC	44	31.43
GNB	78	55.71
Fungi	18	12.86
Total	140	100

Out of 140 culture positive results, bacterial isolates predominate i.e., 122 bacterial isolates (87.14%) and 18 fungi were isolated (12.86%). 78 Gram Negative Bacilli (55.71%) and 44 Gram Positive Cocci (31.43%) were isolated.

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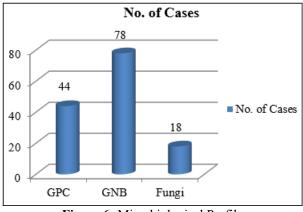


Figure 6: Microbiological Profile

<b>Table 7:</b> Culture Isolates		
Organism	No. of isolates	%
Pseudomonas aeruginosa	52	37.14
Staphylococcus aureus	38	27.14
CoNS	6	4.29
Klebsiella pneumoniae	6	4.29
Proteus mirabilis	6	4.29
Acinetobacter baumannii	6	4.29
Proteus vulgaris	4	2.86
Escherichia coli	4	2.86
Aspergillus niger	12	8.57
Aspergillus flavus	2	1.43
Candida tropicalis	3	2.14
Candida glabrata	1	0.7
Total	140	100

Out of 18 fungal isolates, *Aspergillus niger* predominates i.e., 12 isolates (8.57%), followed by 3 *Candida tropicalis* (2.14%), 2 *Aspergillus flavus* (1.43%) and 1 *Candida glabrata* (0.7%).

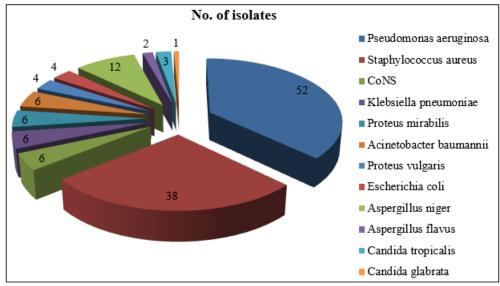


Figure 7: Culture Isolates

Out of 122 bacterial isolates the most commonly isolated organism was *Pseudomonas aeruginosa* i.e., 52 isolates (37.14%), followed by 38 *Staphylococcus aureus* (27.14%). The other organisms isolated were 10 Proteus species (7.15%), 6 CoNS (4.29%), 6 *Klebsiella pneumoniae* (4.29%), 6 *Acinetobacter baumannii* (4.29%) and 4 *Escherichia coli* (2.86%).

 Table 8: AST of Pseudomonas aeruginosa

Drug	Sensitive %	Resistant %
Ceftazidime	44.23	55.77
Gentamycin	71.15	28.85
Ciprofloxacin	84.62	15.38
Amikacin	86.54	13.46
Piperacillin-Tazobactam	88.46	11.54
Meropenem	100	0

Out of the 52 *Pseudomonas aeruginosa* isolates, maximum resistance was noted for ceftazidime (55.77%), followed by Gentamycin resistance (28.85%). The sensitivity of the organisms to Ciprofloxacin, Amikacin and Piperacillin-Tazobactam were 84.62%, 86.54% and 88.46% respectively.

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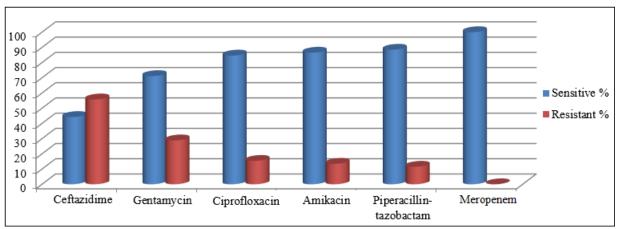


Figure 8: AST of Pseudomonas aeruginosa

All 52 Pseudomonas aeruginosa isolates showed 100% sensitivity to Meropenem.

Table 9: AST of Staphylococcus aureus

Drug	Sensitive %	Resistant %
Penicillin	21.1	78.9
Cotrimoxazole	47.37	52.63
Erythromycin	60.53	39.47
Clindamycin	84.21	15.79
Ciprofloxacin	84.21	15.79
Cefoxitin	89.47	10.53
Vancomycin	100	0

Out of the 38 Staphylococcus aureus isolates, maximum resistance i.e. 78.9% was noted for Penicillin, followed Cotrimoxazole (52.63%) and Erythromycin (39.47%).

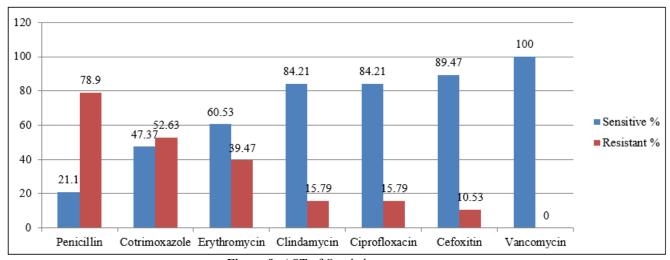


Figure 9: AST of Staphylococcus aureus

The sensitivity of the organisms to Clindamycin and Ciprofloxacin was 84.21%. All showed 100% sensitivity to Vancomycin.

Table 10: MRSA isolates

MRSA	Number	%
Positive	4	10.53
Negative	34	89.47
Total	38	100

Among the 38 Staphylococcus aureus isolates 4 were resistant to Cefoxitin i.e., 4 were MRSA isolates (10.53%).

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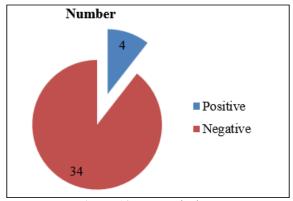


Figure 10: MRSA isolates

#### **Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### **Approval Certificate**



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