



A Cross Sectional Study of Bacterial Etiology of Chronic Suppurative Otitis Media in A Tertiary Care Hospital

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ABSTRACT

Chronic suppurative otitis media (CSOM) is a chronic inflammation of the middle ear, characterized by recurrent ear discharge through a permanent perforation of tympanic membrane. If left untreated, it can lead to serious intracranial as well as extracranial complications. Identifying the etiological agents and their antibiogram is of utmost important in the proper management of the cases. The aim of the study was to identify Bacterial isolates associated with CSOM and their Antibiogram. The study included 200 CSOM

patients clinically diagnosed in the ENT out patient department and 50 swabs from dry ears as controls. samples were sent to Microbiology laboratory where processing was done and bacterial agents were isolated and identified as per standard protocols. Antimicrobial susceptibility testing was performed by Kirby- Bauer’s disc diffusion method. Out of 200 samples, 188 were culture positive. Males were predominant and commonest age group affected was 1-10yrs. 84.40% bacterial agents and 15.60% fungal

agents were isolated. The study identified predominance of Gram-negative bacteria (55.96%). *Pseudomonasaeruginosa* (40.22%) topping the list followed by *Staphylococcus aureus* (20.67%). Incidence of multidrug resistant strains includes, ESBL producers (11.46%), MBL producers (5.04%) and Methicillin resistant strains (12.38%). Maximum sensitivity was observed for Amikacin. Continuous surveillance of etiological agents of CSOM and their antibiogram is necessary to monitor antimicrobial resistance and to guide in empirical treatment.

Keywords

Pseudomonas aeruginosa; *Staphylococcus aureus* ; ESBL ; MRSA ; MBL; Vancomycin.

INTRODUCTION

Chronic suppurative otitis media is a chronic condition, where there is accumulation of purulent fluid in middle ear in addition to tympanic membrane defect¹. It is an important cause of preventable hearing loss in many developing countries². The study of organisms commonly associated with chronic suppurative otitis media is necessary to enable the otologist in management and prevention of complications³. Chronic suppurative otitis media has innumerable complications and Multi-drug resistance³. The indiscriminate use of both topical and systemic antibiotics has led to drug resistance and biofilm formation⁴.

Increasingly resistant isolates are seen in both community and hospitalized patients which render their treatment difficult⁵. *Pseudomonas aeruginosa* strains which produces metallo-beta lactamases have been adversely affecting clinical outcomes and adds to treatment cost^{5,6,7,8}. Hence, the present work aims to isolate and identify the aerobic bacterial isolates of chronic suppurative otitis media, to determine

antibiogram of bacterial isolates, and to detect drug resistance of the bacterial isolates by phenotypic methods.

MATERIALS AND METHODS

The samples were collected from CSOM patients of both sexes and of different ages attending Government Ear, Nose, and Throat Hospital, Out-Patient Department and processed in Department of Microbiology, Andhra Medical College, Visakhapatnam. A prospective study was conducted for a period of 1 year from June 2018 to May 2019 on 200 clinically diagnosed CSOM patients with ear discharge complaint for more than 3 months. 50 ear swabs were collected from patients with healthy ear as controls.

Inclusion Criteria:

- Patients who were not on any antibiotic treatment for previous 48 hours.
- Diagnosed Chronic suppurative otitis media cases of all age groups with chronic ear discharge of at least 3 months period.

Exclusion Criteria:

- Patients currently on treatment with antibiotics and antifungal drugs.
- Patients with acute ear discharge and discharge with less than 3 months duration.
- Patients having Otitis externa with Chronic suppurative otitis media.
- All known HIV or immunosuppression patients.

Collection of Samples

The discharge was collected by sterile swab by gently introducing into the ear under direct visualization and the pus specimen was collected. Two pus samples were collected per ear and placed into the sterile containers which were labelled. The samples were

immediately transported to Laboratory for microbiological isolates.

Processing of Samples

The samples were carried out in the Department of Microbiology, Andhra medical college, Visakhapatnam. 1st swab was used for Gram stain to see the presence of pus cells, morphology of bacilli. 2nd swab was inoculated on Blood agar, MacConkey's agar and Nutrient agar, and incubated at 37°C for 24hrs and observed for growth. After incubation, the colonial and cultural characteristics of isolates were observed, biochemical tests done for identification and documented as per Clinical and Laboratory Standards Institute guidelines⁹.

The antimicrobial susceptibility testing was done by the Kirby Bauer disc diffusion method¹⁰. The Mueller-Hinton agar plates with growth suspension equivalent to 0.5 McFarland standards were incubated at 37°C overnight and the zones were measured as per CLSI guidelines⁹. Methicillin resistance in staph aureus was detected using Cefoxitin 30µg disc. Potentiated Disc Diffusion test (PDT) was used for ESBL and MBL detection in Gram- negative bacteria¹¹. The resistance patterns were further determined by E-test by interpreting Minimum inhibitory concentration (MIC) values (mcg/ml)^{12,11}.

RESULTS

Totally 250 ear swabs from CSOM patients were studied including study group (200 cases) and control group (50 cases). Among 200 cases (study group), 118 were males and 82 were females, which show the incidence of CSOM was higher in males compared to females. Out of 200 ear swabs from chronic suppurative otitis media cases, 188 (94%) samples showed growth, and 12 (6%) samples showed no growth (Table 1.). In the control group, out of 50 ear

swabs, 28 (56%) showed growth in culture and 22 (44%) were culture negative. Out of 28 culture positive samples, CONS 11(39.28%) was the predominant isolate followed by Micrococci 10 (35.71%), Diphtheroid 6 (21.42%) and *C. tropicalis* 1 (3.57%) (Table 2.). In the present study, CSOM incidence was highest between 1 – 10 years age group may be due to frequency of Upper respiratory tract infections (Fig. 1.). Further, the figure 2 shows Male preponderance in CSOM cases and left ear (60.5%) was more affected when compared to right ear (34%) and both ears (5.5%). And the study showed predominance of cases in rural population (63%) when compared to urban population (37%) (Fig. 3.). Out of 188 culture positive cases, 158 (84.05%) cases showed monomicrobial growth. 30 (15.95%) cases showed polymicrobial growth (Table 3.). Total number of isolates obtained from monomicrobial and polymicrobial samples was 218. Out of 218 isolates, bacterial species isolated were 184 (84.40%) and fungal species isolated were 34 (15.60%). Out of total 184 bacterial isolates, 128 were from in pure growth and 56 were from mixed growth (Table 4.). The predominant bacterial isolate was *Pseudomonasaeruginosa* 74 (40.22%) followed by *Staphylococcus aureus* 38 (20.67%), *Proteus* species 24 (13.05%), CONS 20 (10.86%), *Klebsiella pneumoniae* 14 (7.61%), *E. coli* 6 (3.26%), *Acinetobacter baumani* 4 (2.17%), *Micrococci* 2 (1.08%), and *Corynebacterium* species 2 (1.08%). Out of total 34 fungal isolates, the predominant isolate was *Candida albicans* 12 (35.29%), *Aspergillus niger* 12 (35.29%) followed by *Aspergillus flavus* 6 (17.64%) (Table 4.).

Out of 30 mixed infections, 60 isolates were reported. The most common combination of organisms was

Pseudomonasaeruginosa and *Proteusmirabilis* was seen in 6 (20%) samples, *Pseudomonasaeruginosa* and *Klebsiellapneumoniae* seen in 6 (20%) samples (Table 5.).

Most of Gram-negative isolates are sensitive to Imipenem, Amikacin, Piperacillin+ tazobactam, Ceftazidime + clavulanic acid, Ciprofloxacin and Gentamycin (Table 6.). Most of Gram-positive isolates were sensitive to Linezolid, Vancomycin, Teicoplanin, Azithromycin, Amikacin Levofloxacin and Clindamycin (Table 7.). Moreover, in the present study ESBL production was seen in 25 (20.49%) isolates. Out of total 122 Gram-negative isolates, 11 (9.01%) were Carbapenemase producers (Fig. 4 & 5).

Distribution of MRSA strains in Staphylococcus aureus (n= 38)

Out of 38 *Staphylococcus aureus* isolates, 18 (45%) were Methicillin Resistant *Staphylococcus aureus* (MRSA) and 20 (55%) were Methicillin Sensitive *Staphylococcus aureus* (MSSA) (Fig. 6).

Distribution of MDR strains among total isolates (n= 218)

Out of 218 microbial isolates, Multi drug resistant (MDR) strains were 63 (28.89%). Among total Multi drug resistant strains, MRSA isolates were 27 (12.38%), ESBL producers were 25 (11.46%) and Carbapenemase (MBL) producers were 11 (5.04%) (Table. 8).

Therapeutic Response (n= 188)

Out of total 188 cases, 140 recovered with antibiotics, 32 showed no response to treatment and 16 were defaulters (Fig. 7).

DISCUSSION

The study evaluated the aetiological agents (bacterial & fungal) of CSOM and their antimicrobial susceptibility. Males (59%) were more affected than

females (41%) in the present study which correlated with Narayana R.S. et. al.¹³ (59%), N. Lakshmi (58.9%)¹⁴, Saranya SK. et. al.¹⁵ (61.42%) Raghu Kumar KG et. al.¹⁶ (62.26%) Whereas, Ramakrishna PJ. et. al.¹⁷ (54.7%), Nikakhlagh S. et. al.¹⁸ (54%) and Sharma M. et. al.¹⁹ (52%) reported slightly lower incidence.

CSOM cases were predominantly seen between the age group of 1-10 yrs (42.5%) which correlates with the study of N. Lakshmi (46.1%)¹⁴, Sarathbabu (43.75%)²⁰ and Bhumbla U. et. al. (38.5%)⁵, Vaidya K et. al.²¹ (28.57%) and Sharma M. et. al.¹⁹ (24.8%). Whereas the studies of Kumar H. et. al.²² (35.71%), Prakash et. al.²³ (26.47%) and Sagar Kashyap et al.²⁴ (42.16%) reported higher incidence in the age group of 11- 20 years and the study of Raghu Kumar KG et. al.¹⁶ (25.42%) reported higher incidence in 21- 30 years. Studies of Ramya SN et. al.¹³ (37.1%) and Loy AH. et. al.²⁵ (23.3%) reported higher incidence in age group of 31- 40 years.

In the present study, left ear (60.5%) was more affected when compared to right ear which correlates with study of Sarath babu R. (61.25%)²⁰, Saranya SK. et. al.¹⁵ (60%) Kamal N et. al.²⁶ (46.7%) and N. Lakshmi (56.6%)¹⁴ Whereas, Ramakrishna PJ et. al.¹⁷ (32%) and Hirapure PV et. al.²⁷ (33.8%) reported less incidence in left ear.

Majority of CSOM cases were from rural areas (63%) which correlates with the study of Sarath babu R. (66.25%)²⁰, Harshika YK. et. al.²⁸ (60%) and Kaur P. et. al.²⁹ (59%) Whereas, Sharma M. et. al.¹⁹ (72%), Kumar H. et. al.²² (75%), Harrison Phiri. et. al.³⁰ (81%) and Bhumbla U. et. al.⁵ (81.91%) reported higher incidence due to low socio-economic status, lack of personal hygiene and poor education.

Analysis of the 200 samples of CSOM revealed that culture positivity was seen in 94% of the cases. This correlates with study of Loy AH et.al.²⁵ who reported (96.5%), Pajor A. et.al.³¹ (96.5%), Sangeeta Baskaran³² (93%), Shashidhar V. et.al.³³ (88.5%), Bhumbala U. et.al.⁵ (88.5%) and Sharma M. et.al.¹⁹ (91.2%). Monomicrobial growth seen in 84.05%, mixed growth was seen in 15.95% in the present study which coincides with Sarath babu (83.2% & 16.8%)²⁰, Harrison phiri et al³⁰ (85.8% & 14.2%) and Pajor et. al.³¹ (82.5% & 17.5%).

In the study, 78.40% of bacterial isolates were reported which coincides with the studies of Fatima G et. al.³⁴ who reported 80.07%, Bhumbala. U. et. al.⁵ (79%). Shashidhar V. et. al.³³ (74.8%), Saranya SK et. al.³⁵ (69.79%), and Kumar H et.al.²² (69%). Whereas, Narayana R. S. et. al.¹³ (62.4%) and Attalah MS. et. al.³⁶ (48%) reported relatively lower incidence. A higher incidence was reported by Sharma M. et.al.¹⁹ (89.6%), Pajor A. et. al.³¹ (88.6%), Loy AH et. al.²⁵ (87.7%) and Sangeeta Baskaran³² (87%). Most predominant agent was *Pseudomonas aeruginosa* (40.22%) followed by *Staphylococcus aureus* (20.67%), which coincides with the study of Attallah MS et al.³⁶ (51.7%), Wariso BA et. al.³⁷ (41%), Malkappa SK et. al.³⁸ (45.2%), and Raghu Kumar KG et. al.¹⁶ (42.2%) Fatima G et. al.³⁴, Raghu Kumar KG et. al.¹⁶ and Sagar Kashyap et al²⁴. whereas, Vaidya K et. al.²¹ (54.5%) and GH. Ettehad et al.³⁹ (31.15%) reported *Staphylococcus aureus* as the predominant isolate followed by *Pseudomonas aeruginosa* in their studies.

Imipenem (90%) followed by Amikacin (85%) and Piperacillin+ tazobactam (89%) showed highest sensitivity for Gram-negative isolates which coincides with study of Saranya SK et al.¹⁵, Harrison Phiri et.

al.³⁰, Fatima G et. al.³⁴ and Raghu Kumar KG et. Al³⁰.

Pseudomonas aeruginosa isolates were sensitive to Colistin (88%), Imipenem (82%), Aztreonam (92.4%), Amikacin (91%), Piperacillin+ Tazobactam (92%), Ceftazidime (77%) which coincides with study of Saranya SK et al¹⁵, Fatima G. et. al.³⁴ and Soumya S. et. al.⁴⁰ Vancomycin (100%) and Amikacin (89%) were highly active against gram positive isolates which correlated with study of Saranya SK et. al.¹⁵, Sagar Kashyap et. al.²⁴ and Kumar H. et. al.²²

Among the total CSOM isolates 218, Multi drug resistant strains were 63 (28.89%). Out of them, MRSA isolates were (12.38%) which correlated with study of S. Nanarayan R et. al.¹³ (11.79%), Ramakrishna PJ et. al.⁴¹ (13.63%) and Fathy Mohamed ES. Et. Al⁴³ (10.83%). Bhumbala U. et. Al⁵ reported 33.33%. Among 122 Gram- negative isolates, (20.49%) were ESBL producers which is similar to the studies of Prasanth D.P. et. al.⁴⁴ (22.22%), Ramesh A. et. al.⁴² (24.5%). Whereas Sagar Kashyap et.al.²⁴ (62.5%), Rejitha I.M. et. Al⁴⁵ (31.57%) and Bhumbala U. et. al.⁵ (33.33%) reported higher incidence of ESBL producers. Fathy Mohamed E.S. et. al.⁴³ (3.7%) and Sattar A et. al.⁴⁶ (6.6%) reported lower incidence of ESBL producers respectively. Metallo beta Lactamases were (9.02%) similar to that of Chakraborty B. et. al.⁶ (10.71%), Ramesh A. et. al.⁴² (6%) and Sagar Kashyap et. al.²⁴ (18.18%). Whereas the studies of Sowmya S. et. al.⁴⁰ (30%), Neelaveni D. et. al.⁴⁷ (29.1%) reported higher incidence and Harshika YK. et. al.⁴⁸ (5.13%) reported relatively lower incidence

CONCLUSIONS

The study showed that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the predominant bacterial

isolates in CSOM patients. Antibiotic susceptibility testing identified that most of Gram-negative isolates were sensitive to Imipenem, Amikacin, Piperacillin+tazobactam, Ceftazidime + clavulanic acid, Ciprofloxacin, and Gentamycin. Most of Gram-positive isolates were sensitive to Linezolid, Vancomycin, Teicoplanin, Azithromycin, Amikacin and Clindamycin. Recently, the multidrug resistant strains are increasing gradually due to injudicious use of drugs, availability of over-the-counter drugs and increase use of steroid drops. Majority of cases responded to antibiotic treatment. No response in few cases could be mainly, due to development of cholesteatoma or mastoiditis which needs surgical treatment.

Thus understanding the etiology and local antibiogram pattern of CSOM isolates is extremely helpful for the clinical in choosing the appropriate empirical antibiotics which further prevent the emergence of resistance strains.

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Table 1: Culture positivity among Total Samples (n= 200)

Samples	Number of cases	Percentage
Culture positive samples	188	94%
Culture negative samples	12	6%
Total samples	200	100%

Table 2: Organisms Isolated in Control Group (n= 28)

S.No	Isolate	No	%
1.	Coagulase negative Staphylococci	11	39.28%
2.	Micrococci	10	35.71%
3.	Corynebacterium species (diphtheroid)	6	21.42%
4.	Candida tropicalis	1	3.57%
	TOTAL	28	100%

Table 3: Prevalence of Pure and Mixed Infections (n= 188)

Culture positivity	Number	Percentage
Monomicrobial (Pure)	158	84.05%
Polymicrobial (Mixed)	30	15.95%
Total	188	100%

Table 4: Distribution of Bacterial Isolates in Pure and Mixed cultures (n=184)

Organism	Total		Pure		Mixed	
	No.	%	No.	%	No.	%
<i>Pseudomonas aeruginosa</i>	74	40.22%	56	30.43%	18	9.78%
<i>Staphylococcus aureus</i>	38	20.67%	26	14.13%	12	6.52%
<i>Proteus</i> species	24	13.05%	10	5.43%	14	7.60%
<i>Proteus mirabilis</i>	(16)		(6)		(10)	
<i>Proteus vulgaris</i>	(8)		(4)		(4)	
Coagulase negative Staphylococci	20	10.86%	16	8.69%	4	2.17%
<i>Klebsiella pneumoniae</i>	14	7.61%	8	4.34%	6	3.26%
<i>Escherichia coli</i>	6	3.26%	6	3.26%	-	-
<i>Acinetobacter baumannii</i>	4	2.17%	2	1.08%	2	1.08%
Micrococci	2	1.08%	2	1.08%	-	-
<i>Corynebacterium</i> species	2	1.08%	2	1.08%	-	-
TOTAL	184	100%	128	69.56%	56	30.43%

Table 5: Combination of Organisms in Mixed cultures (n=30)

Bacteria & Fungi or more than one Bacteria	No. of samples	No. of isolates
<i>Pseudomonas aeruginosa</i> + <i>Proteus mirabilis</i>	6	12
<i>Pseudomonas aeruginosa</i> + <i>Klebsiella pneumoniae</i>	6	12
<i>Pseudomonas aeruginosa</i> + <i>Staphylococcus aureus</i>	4	8
<i>Proteus vulgaris</i> + <i>Staphylococcus aureus</i>	4	8
<i>Proteus mirabilis</i> +Coagulase negative Staphylococci	4	8
<i>Acinetobacter baumannii</i> + <i>Staphylococcus aureus</i>	2	4
<i>Pseudomonas aeruginosa</i> + <i>Aspergillus niger</i>	2	4
<i>Staphylococcus aureus</i> + <i>Candida albicans</i>	2	4
TOTAL	30	60

Table 6: Antibiotic sensitivity pattern of Gram-positive Isolates (n=122)

Organisms	AMP	CIP	AK	GEN	CAZ	IMP	PIT	CAC	CTX	AT	CO
<i>P. aeruginosa</i>	24%	35%	91.5%	60%	77%	82%	92%	80%	35%	92%	88%
<i>E. coli</i>	35%	70%	85%	68%	65%	90%	100%	90%	65%	NT	NT
<i>K. pneumoniae</i>	25%	85%	75%	65%	75%	85%	90%	90%	50%	NT	NT
<i>Proteus spp</i>	20%	20%	80%	60%	50%	95%	98%	92%	57%	NT	NT
<i>A. baumannii</i>	-	-	25%	25%	50%	100%	75%	75%	25%	NT	NT

Table 7: Antibiotic sensitivity pattern of Gram -positive Isolates (n=62)

Organism	AMP	AK	TEI	CD	CTX	CX	LZ	CIP	VA	AZ	LE
<i>S. aureus</i>	74%	89%	100%	85%	65%	55%	97%	42%	95%	93%	79%
CONS	42%	78%	100%	78%	55%	42%	100%	58%	100%	85%	78%

Table 8: Distribution of MDR strains among total isolates (n= 218)

Total isolates		MDR strains		MRSA		ESBL producer		MBL producers	
No.	%	No	%	No	%	No.	%	No.	%
218	100%	63	28.89%	27	12.38%	25	11.46%	11	5.04%

Figure Legends

- Figure 1: Age and sex wise distribution of CSOM cases.
- Figure 2: Ear wise distribution of cases among both sexes.
- Figure 3: Distribution of cases based on Demographic area. (n=200)
- Figure 4: ESBL producers among various gram –negative isolates (n = 25)
- Figure 5: MBL producers among various gram- negative isolates (n = 11)
- Figure 6: Distribution of MRSA strains *S.aureus* (38).
- Figure 7: Therapeutic response of patients.

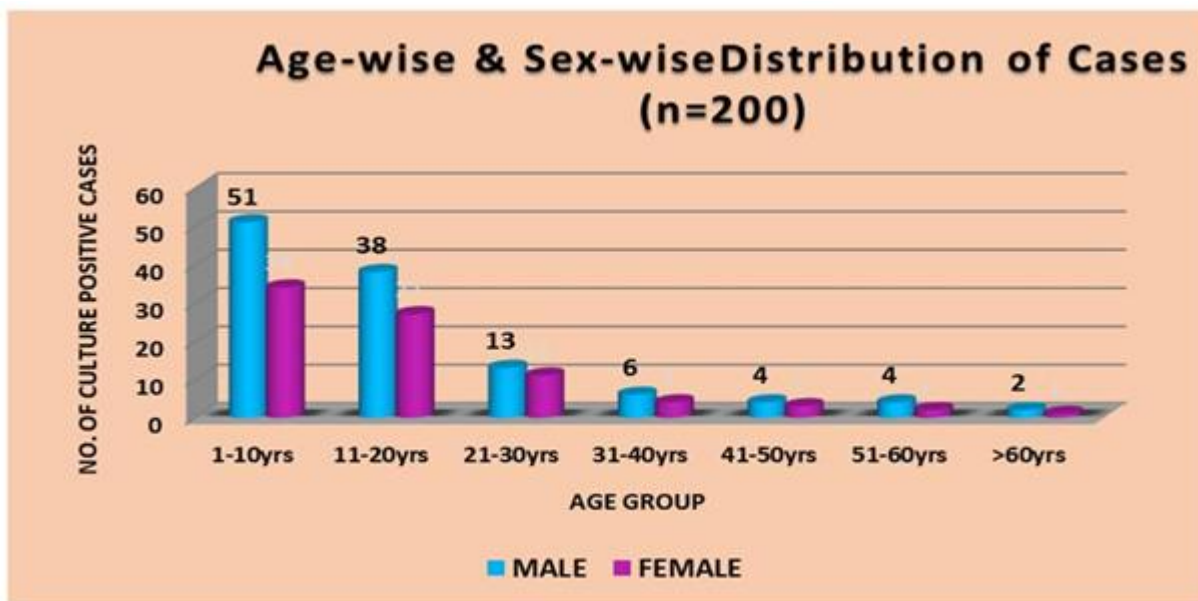


Figure 1. Age and sex wise distribution of CSOM cases.

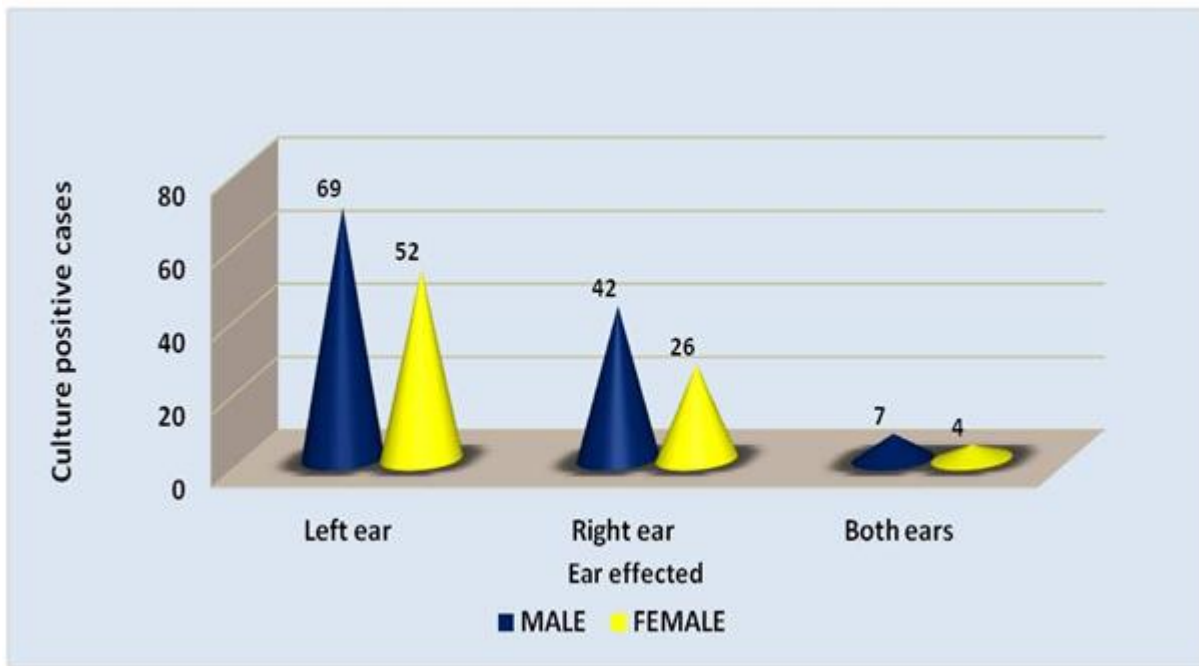


Figure 2. Ear wise distribution of cases among both sexes.

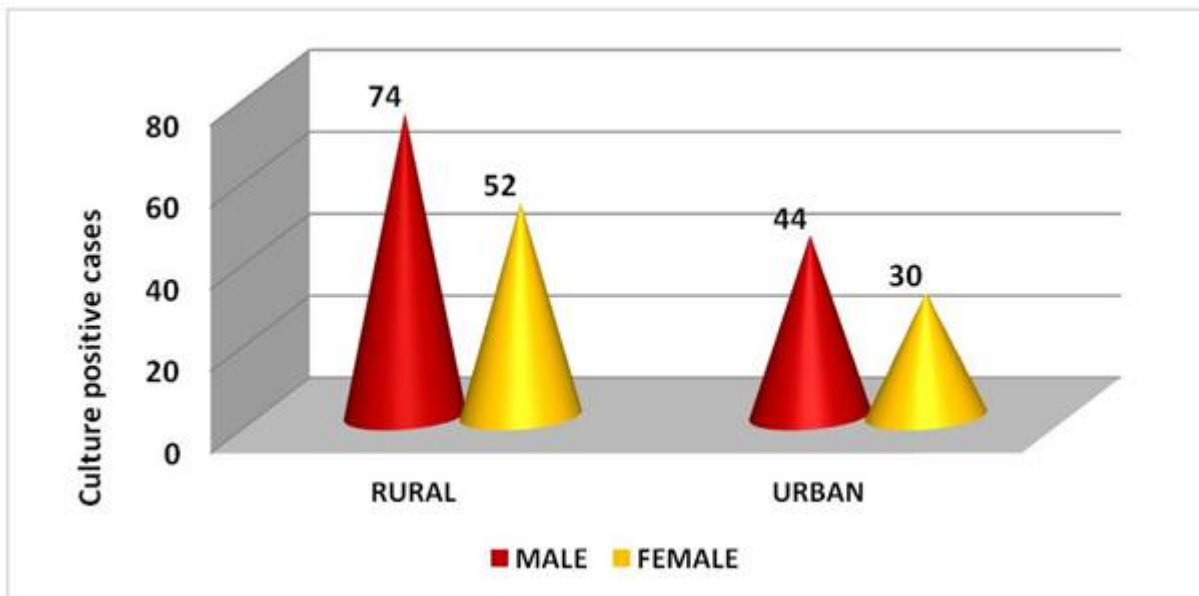


Figure 3. Distribution of cases based on Demographic area. (n=200)

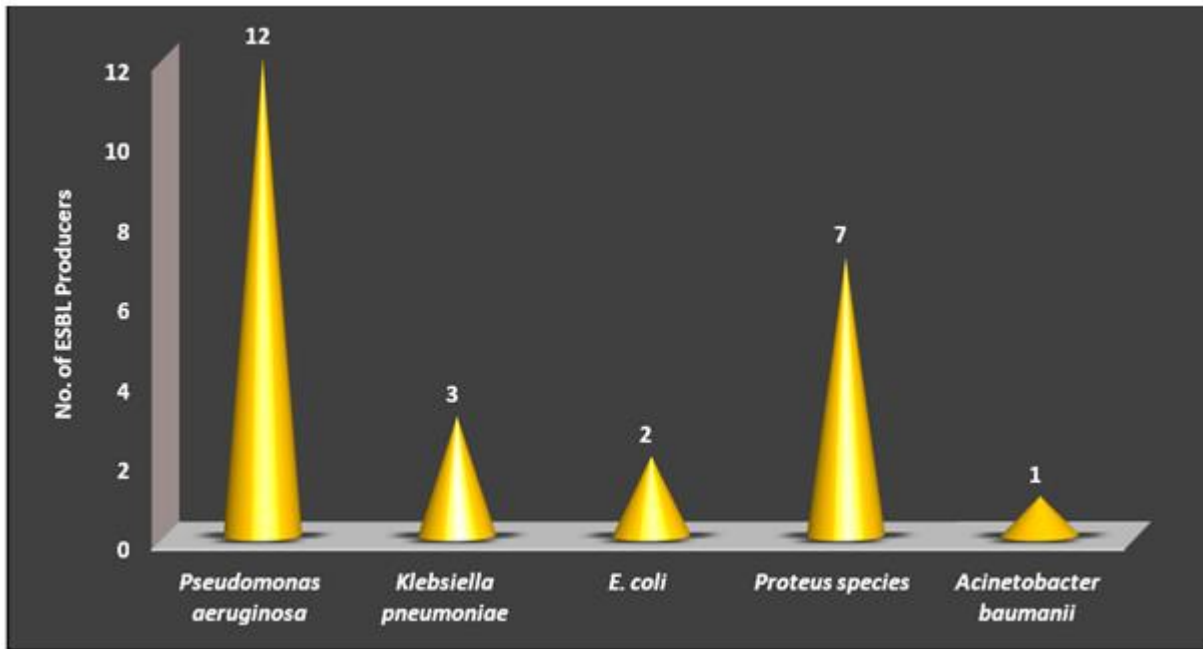


Figure 4. ESBL producers among various gram –negative isolates (n = 25)

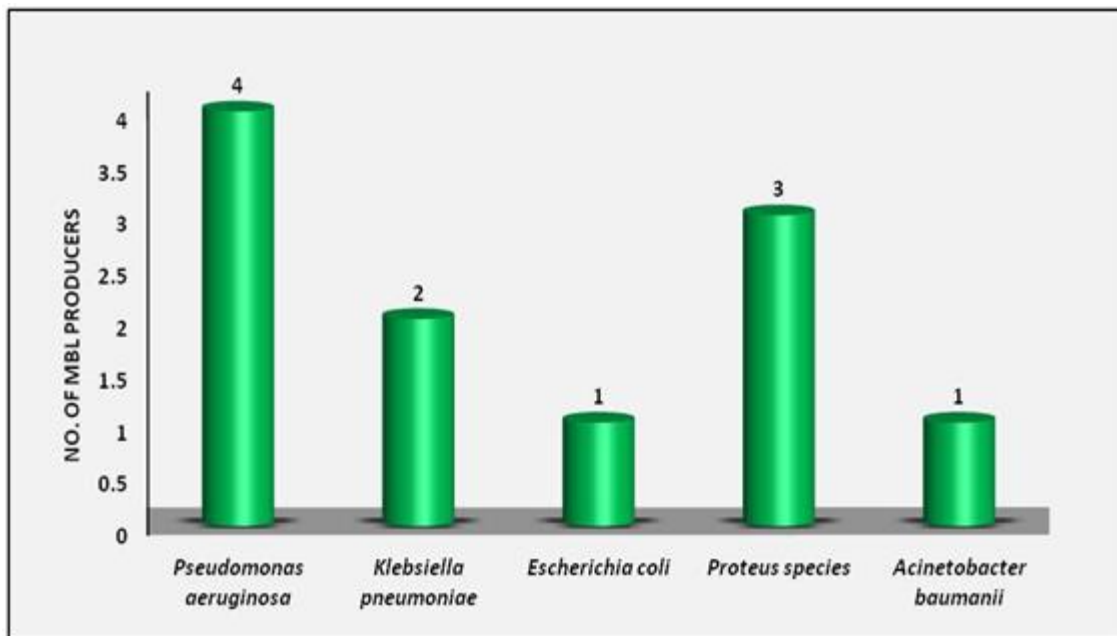


Figure 5. MBL producers among various gram- negative isolates (n = 11)

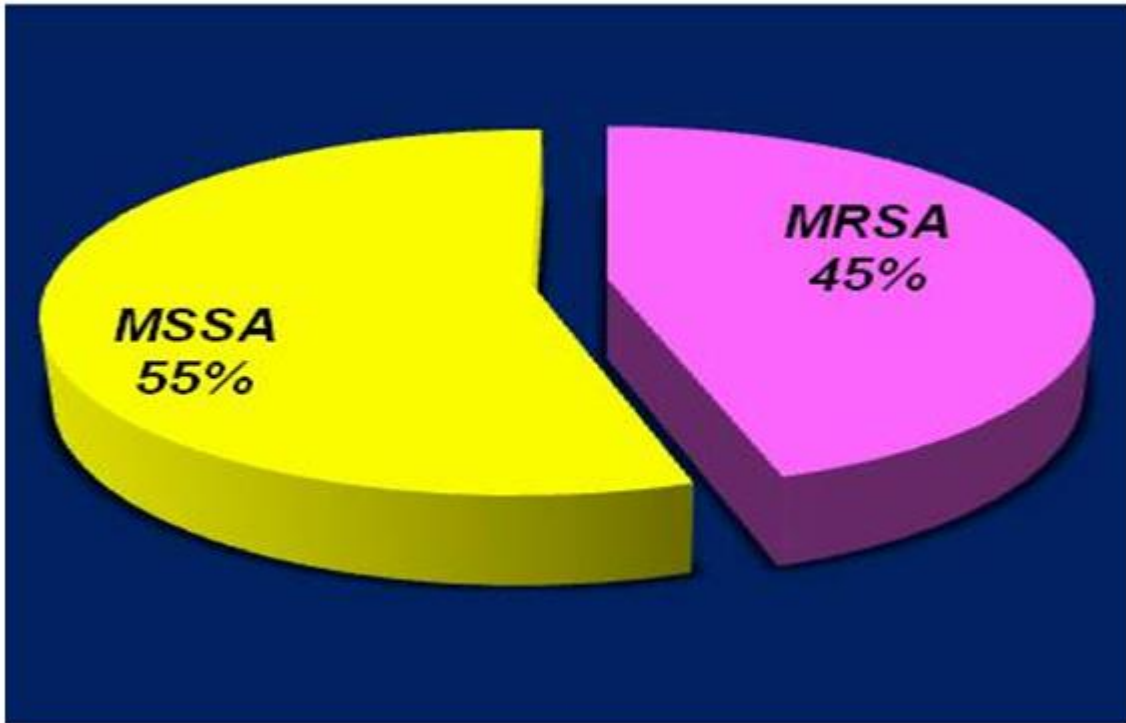


Figure 6. Distribution of MRSA strains S.aureus (38).

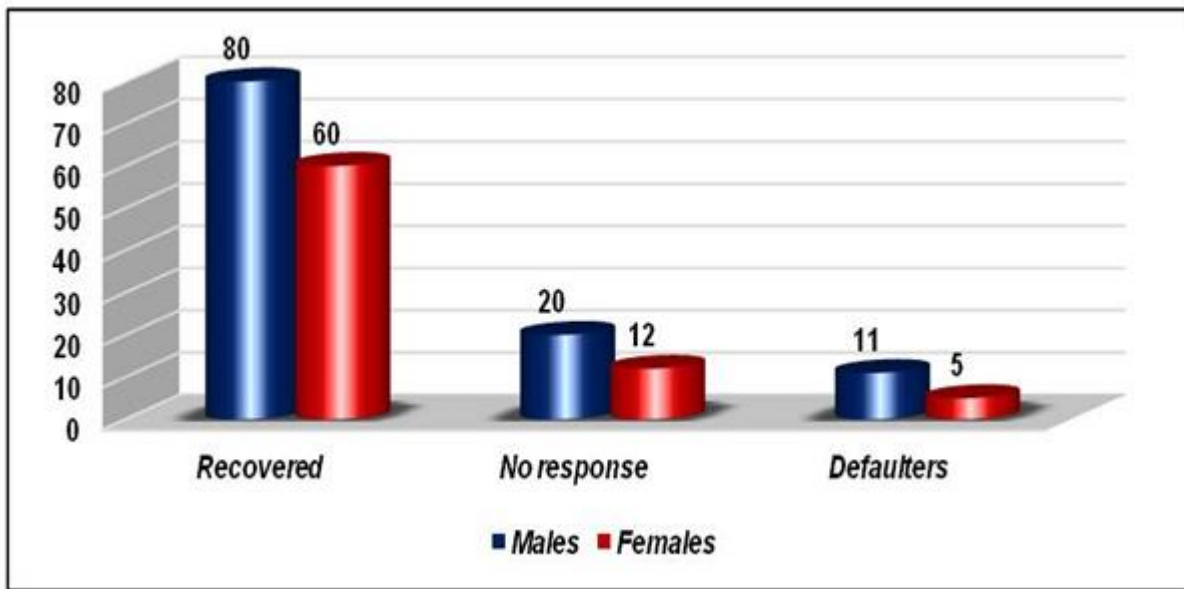


Figure 7. Therapeutic response of patients.