ORIGINAL RESEARCH

Antimicrobial susceptibility analysis of bacterial culture positive isolates at teaching tertiary care hospital in Gujarat

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ABSTRACT

Background: To understand the constantly changing pattern of microbial drug resistance it is crucial to have data analysis of various drug-bug susceptibility trends. In view of rising concern of antimicrobial resistance across the globe it is of prime importance to analyze local susceptibility trends and in turn to draw the local guidelines about the antimicrobial usage based on its outcome. This study aims to analyze the prevalent susceptibility patterns and identify the rate of resistant organisms among the bacterial culture positive isolates at the teaching tertiary care hospital in Gujarat in the year 2022. Total of 2754 clinical samples were sent to the bacteriology laboratory for the culture & sensitivity analysis during the period of one year, out of which 908(32.97%) were found to be culture positive where as 1846 (67.02%) were found to be culture negative. Out of 908 culture positive specimens the gram positive organisms were identified in 278(30.61%) isolates and gram negative organisms were identified in 630(69.38%) isolates. Further analysis of these isolates showed that among the gram negative strains, *Escherichia coli* (*E.coli*) was the predominant organism with total number of 291(32.04%) isolates, followed by *Klebsiella spp* with total number of 105(11.56%) isolates. Whereas among the gram positive strains, *Staphylococcus aureus* was the predominant organism with total number of 137(15.08%) isolates, followed by the *Coagulase Negative Staphylococcus spp* (*CoNS*) with total number of 78(8.59%) isolates. Inaddition this study also showed that there was significant number of isolates having resistant profiles including β lactamases, *Methicillin resistant staphylococcus aureus* (MRSA), and *Carbapenemresistant Enterobacteriaceae* (CRE). This analysis helps to understands the prevailing or developing resistant trends in comparison to the similar studies done in other parts of India and world

Keywords – Antimicrobial susceptibility, Clinical specimens, Bacterial isolates

INTRODUCTION

There is huge risk of increased infection rates which are becoming untreatable due to the rise in the resistant to antimicrobials, and fewer antimicrobials being researched. There is also vast burden of multidrug resistant organisms (MDRO) being isolated from the routine specimens. As the pattern of antimicrobial susceptibility keeps changing from place to place and from time to time, it requires ongoing data analysis for its awareness. As per the article published in The Lancet January19, 2022 there were estimated 4.95 million (between 3.62-6.57) deaths associated with bacterial AMR in 2019 alone.[1] A five-year antimicrobial susceptibility trends study done at hospital in Rwanda Africa, found the high rates of resistance by gram negative bacteria to cephalosporins and rising rates of resistance to carbapenems and colistin indicating the need for AMR surveillance, implementation of antimicrobial stewardship programs and implying the infection control practices to curb this problems.[2]These health care issues highlights the need of identifying the epidemiology and changing trends of antimicrobial susceptibility, in order to implement the health care policy and improve the cost outcome. Two year study done in Denpasar-Bali by Masyeni*et al.*, in 2018 reported rate of MDR isolates as high as 47.7% which included five main isolate types showing resistance to three or more antibiotic classes with various magnitudes.[3]

A retrospective study of patients' blood culture collected over a 7-year period by Gandra S *et al.*, found continual high rates of *Methicillin-resistant Staphylococcusaureus* (MRSA; approximately 44.2%), high resistance to Nalidixic acid among *Salmonella typhi* (98%) and increased Carbapenem resistance in both *Escherichiacoli* (7.8% to 11.5%; p = 0.332) and *Klebsiella pneumoniae* (41.5% to 56.6%; p < 0.001). For *Acinetobacter species* it was approximately 69.6% whereas for *Pseudomonas aeruginosa* it was approximately 49%. Also seen were presence of Colistin resistance in Gram-negative organism, Vancomycin and Linezolid resistance in *S. aureus*. Increasing resistance to antibiotics of last-resort poses an urgent need for new antibiotics and improved antimicrobial stewardship programs in India. [4]

To monitor the emergence of new resistance mechanisms in pathogens, constant check on the susceptibility pattern of an individual organism is required which can only be evaluated by ongoing analysis of the susceptibility reports. This in turn can help to incorporate the antimicrobial stewardship programs at the local levels. This study will also compare the patterns of resistance to various drug-bug combinations.

GOAL/AIM

This study aims to analyze the antimicrobial susceptibility among bacterial culture positive isolates at teaching tertiary care health care facility in Gujarat and to understand the prevailing or newly developing susceptibility patterns among the microorganisms isolated from various clinical specimens.

OBJECTIVES

1. To study the antimicrobial susceptibility trends of bacterial culture positive isolates at teaching tertiary health care facility in state of Gujarat.
2. To compare rate of resistance to various drug-bug combinations with existing or newly identified patterns.

MATERIALS AND METHODS

Study design: Retrospective cross-sectional data analysis.

Study setting: Dr. N. D. Desai Medical College & Hospital, Dharmsinh Desai University, Nadiad, Gujarat.

Study period: January to December 2022

Study samples: All the bacterial culture positive samples received from IPD or OPD sections of Dr. N D Desai Hospital during the period of January to December 2022.

Data collection method: Antimicrobial susceptibility analysis data was collected from the Bacteriology section of the Microbiology department.

STUDY PROCEDURES

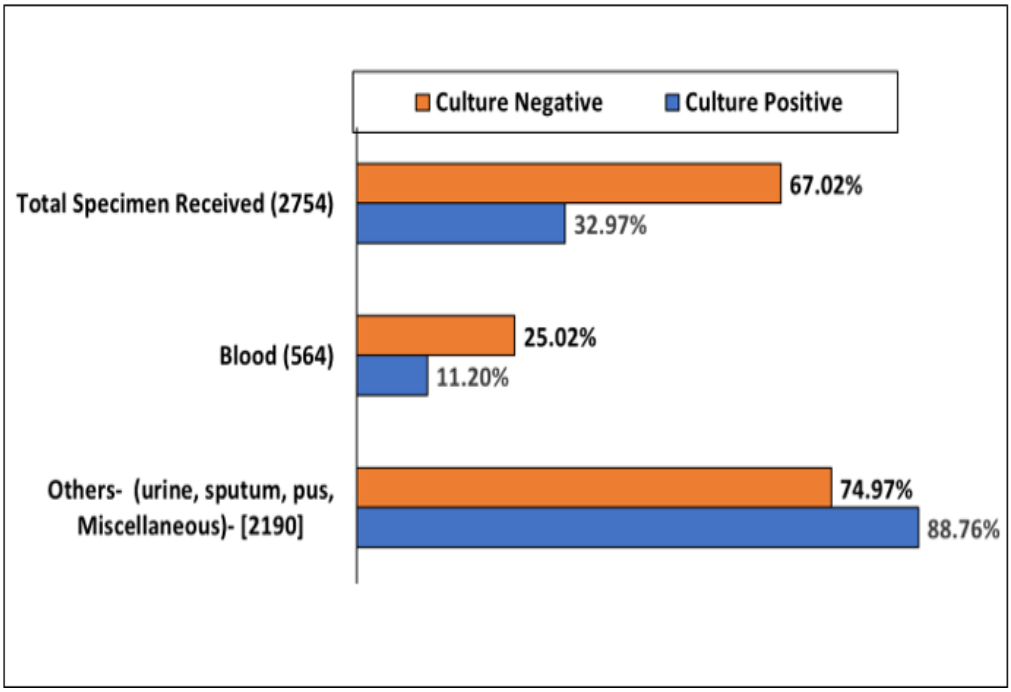
Sample processing, antimicrobial susceptibility analysis and statistical analysis of bacterial culture positive samples:

This study procedure included all the bacterial culture positive samples of all categories (Blood, Urine, Sputum, Pus, Body fluids, Stool, and Miscellaneous) collected by standard microbiological techniques and received at the department of Microbiology, of Dr. N D Desai Hospital during the period of January to December 2022. Samples were processed as per its type and the standard microbiological culture procedures. All the culture growth positive samples were further processed for biochemical reactions for species identification and antimicrobial susceptibility testing (AST) as per the standard CLSI guidelines - 31st edition, 2021.[5] At the end of the AST incubation period sensitivity or resistant patterns were measured using standard CLSI guidelines. Data were statistically analyzed using the Microsoft Excel 2019 software using sort, filter and percentage applications. It was further analyzed for its distribution in the IPD/OPD/ Critical care units area, specimen type, organism groups, and individual antibiotic sensitivity/resistant percentage for the organism groups and isolates. Percentage ratio of the organisms sensitivity/resistant type was also compared with past and recent studies done at different centers. Final analysis report was also submitted to institutional antimicrobial policy committee to help them to implement the antimicrobial stewardship in their policy.

Ethical considerations: Institutional Ethical committee (IEC) approval was taken for the data collection & analysis. (on 12-04-2023).

RESULTS

Table 1: Culture Positive & Culture Negative distribution of total number of samples received.



Total of 2754 samples of various types such as urine, sputum, pus swabs/pus aspirates, body fluids (ascitic/ pleural/synovial), biopsy, tissue, stool, nails, endotracheal tube, tracheal tube, drains, bronchoalveolar lavage, umbilical catheter, prosthesis, endometrial collections etc.. were collected for the bacterial culture and sensitivity analysis were processed as per the microbiological and CLSI standards. Out of which 908 (32.97%) were found to be culture positive and 1846 (67.02%) were reported as culture negative.[**Table 1**] From 908 culture positive samples 806(88.76%) were of various types as above and 102 (11.20%) were blood samples. 802(88.32%) samples were from non-critical care areas where as 106 (11.67%) samples were from critical care areas including NICU, PICU, SICU, ICU. As per **Table 2**, Maximum number of clinical samples were received from the department of surgery followed by ENT, Medicine and TBCD. As shown in the **Table 3** below the highest number of culture positive specimen type received was that of Pus 359 (39.53%) followed by Urine 247(27.20%), Sputum 155(17.07%) and Blood 102(11.23%).

Table 2: Clinical department and location wise distribution of the culture positive samples.

Department	IPD	OPD	TOTAL
Surgery	160	39	199
ENT	15	120	135
Medicine	93	37	130
TBCD	107	3	110
OBGY	16	64	80
Emergency Medicine	61	0	61
Orthopedic	13	28	41
Pediatrics	27	11	38
Ophthalmology	1	4	5
Dermatology	0	3	3
NICU	40	0	40
PICU	20	0	20
SICU	09	0	09
ICCU/ICU	37	0	37
TOTAL	**599**	**309**	**908**

Table 3: Different Specimen type distribution of the culture positive samples.

SPECIMEN DISTRIBUTION	IPD	OPD	TOTAL
Pus /Swabs/Pus aspirates	163	196	359
Urine	154	93	247
Sputum	151	4	155
Blood	90	12	102
Body fluids/ (Ascitic/Pleural/Synovial)	9	5	14
Biopsy/Tissue	13	2	15
Others (Nail, ET, TT, Drain, BAL, Umbilicalcatheter, Prosthesis, Endometrial collection,)	13	1	14
Stool	2	0	2
TOTAL	**595**	**313**	**908**

Further distribution of the organism isolates into specimen type was as per the **Table 4& Table5**. Among the gram negative isolates *Escherichia coli (32.04%), Klebsiella spp (11.56%), Pseudomonas spp (17.62%), Acinetobacter spp (2.20%), Enterobacter spp (1.43%) and Proteus spp (1.76%)* were the major organism type. For gram positive isolates majority were *Staphylococcus aureus (15.08%), Coagulase negative staphylococci (8.59%), Enterococci spp (4.40%) and Streptococcispp* (2.42%)were the major isolates. *E.coli* was major isolate from the Urine samples, where as*Staphylococcus aureus & Pseudomonas* were the major isolates from Pus samples.

Table 4: Major gram negative bacterial isolates from the culture positive samples.

Specimen type	*E.coli*	*Klebsiella spp*	*Pseudomonas spp*	*Acinetobacter spp*	*Entero-bacterspp*	*Proteus spp*	Total
Blood	6	15	4	2	2	0	29
Urine	178	14	14	6	1	3	216
Sputum	37	45	48	9	9	0	148
Pus/swabs/Aspirates	63	26	84	3	0	13	189
Body fluids (Ascitic/Pleural/Synovial)	0	0	4	0	0	0	4
Tissue/Biopsy	3	4	2	0	0	0	9
Others (Miscellaneous)	4	1	4	0	1	0	10
Total Isolates	291	105	160	20	13	16	605

(Miscellaneous-nail/ET/TT/drain/BAL/umbilical catheter/prosthesis/endometrial collection)

Table 5: Major gram positive bacterial isolates from the culture positive samples.

Specimen type	*Staphylococcus aureus*	*Coagulase Negative Staphylococci*	*Enterococci spp*	*Streptococci spp*	Total
Blood	4	43	5	4	56
Urine	9	7	19	0	35
Sputum	4	0	0	3	7
Pus/swabs/Aspirates	104	28	12	15	159
Body fluids (Ascitic/Pleural/Synovial)	8	0	2	0	10
Tissue/Biopsy	6	0	0	0	6
Others (Miscellaneous)	2	0	2	0	4
Total Isolates	**137**	**78**	**40**	**22**	**277**

Further susceptibility analysis of these isolates were done to understand the sensitivity and resistant pattern of the predominant organisms.

For *E.coli* the antimicrobial sensitivity pattern was as per the figure.1 *E.coli* showed higher sensitivity (78.69%) for carbapenem group of antibiotics where as decreased sensitivity for the β lactamases & cephalosporins. [Figure 1]

***Figure 1 to 4: Susceptibility percentage of major gram negative isolates.**

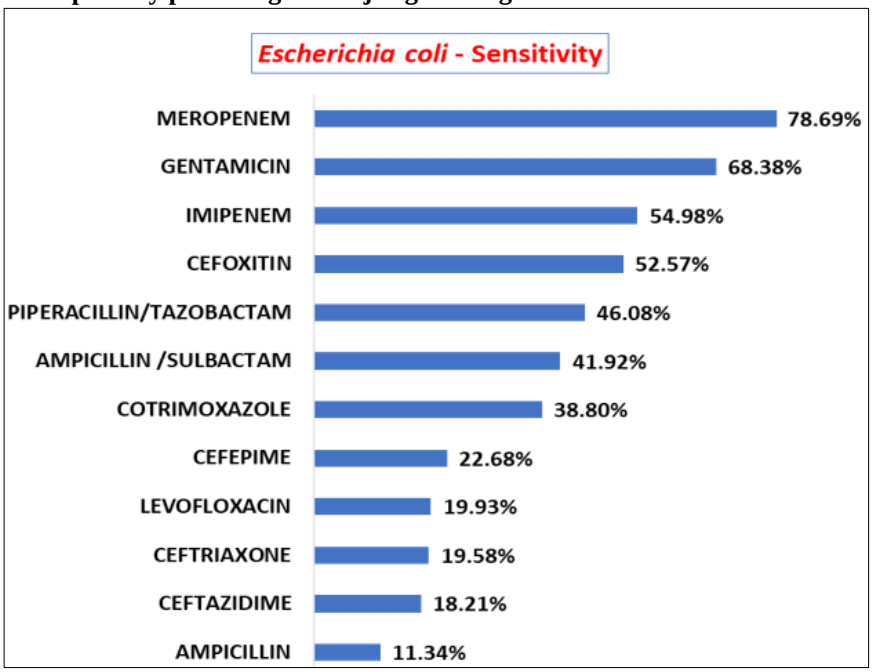


Figure 1

For *Klebsiella spp* antimicrobial sensitivity pattern was as in the figure.2 *Klebsiellaspp*showed higher sensitivity for the Gentamicin (82.85%) where as quinolones and cephalosporins showed decreased sensitivity pattern. [Figure 2]

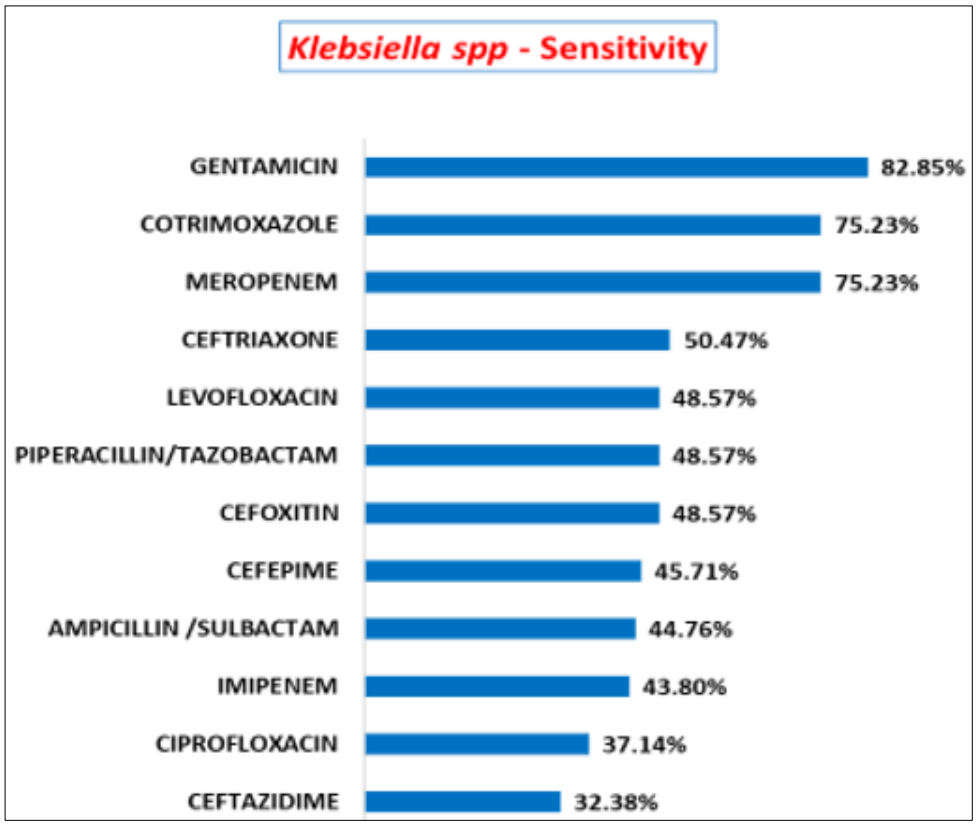


Figure 2

For *Pseudomonas spp* all the tested groups of antibiotic showed sensitivity of more than 50%, where as decrease sensitivity of <50% were seen for most of the tested groups of antibiotics for *Acinetobacter spp* except Piptaz (50%), Levofloxacin(50%), Cotrimoxazole(55%) & Gentamicin(60%). [Figure 3 &4]

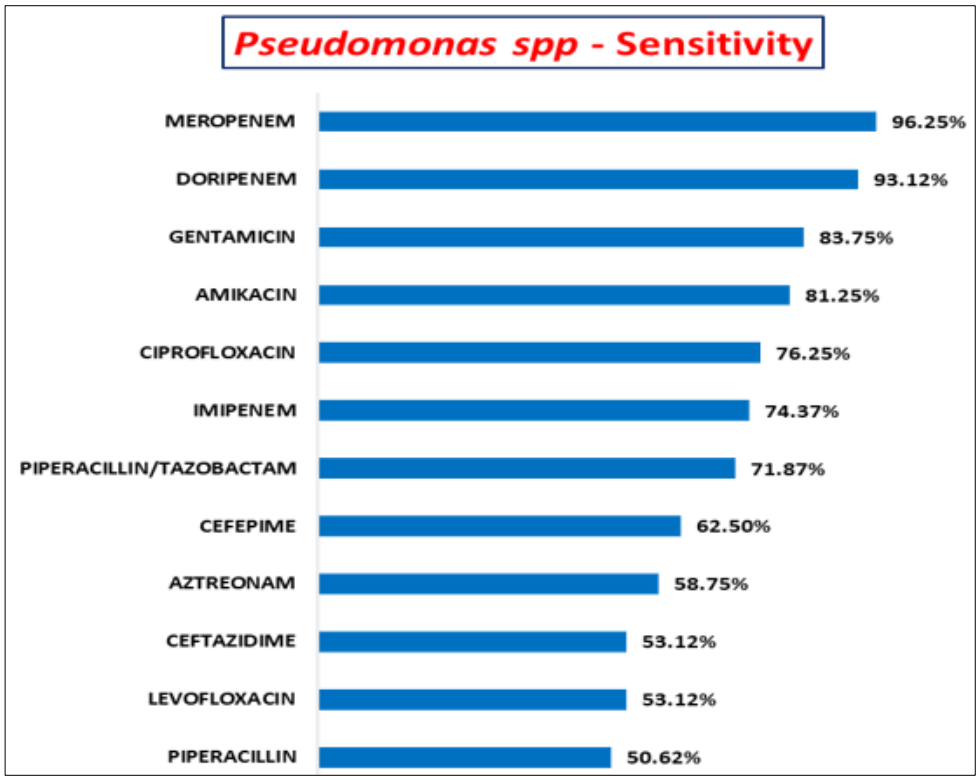


Figure 3

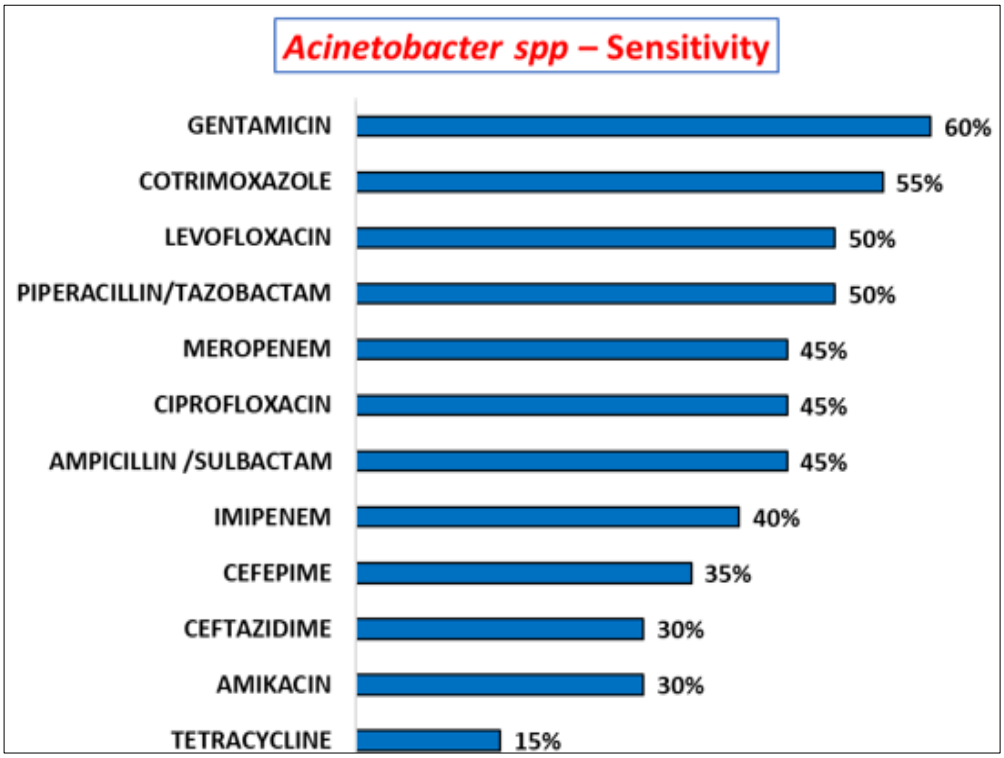


Figure 4

Among the gram positive isolates *Staphylococcus aureus*&*CoNS* sensitivity pattern was as per the **Figure5 & 6.**
***Figure number 5 to 7 Susceptibility percentage of major gram positive isolates.**

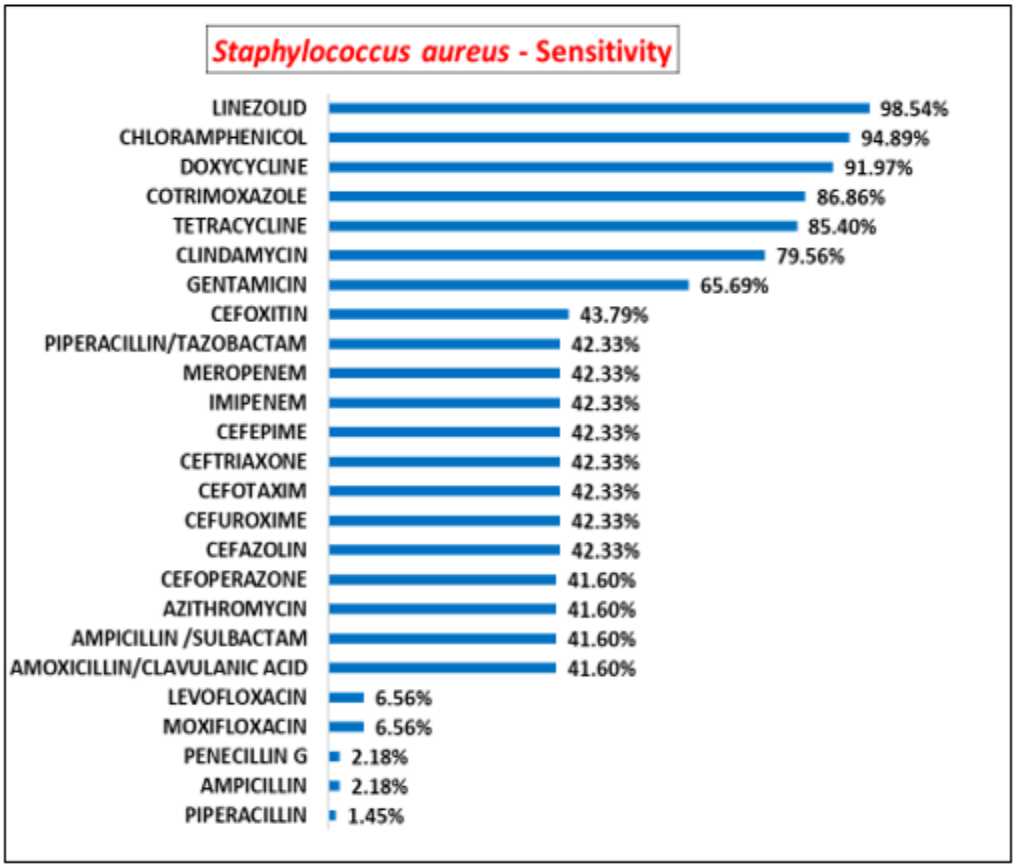


Figure 5

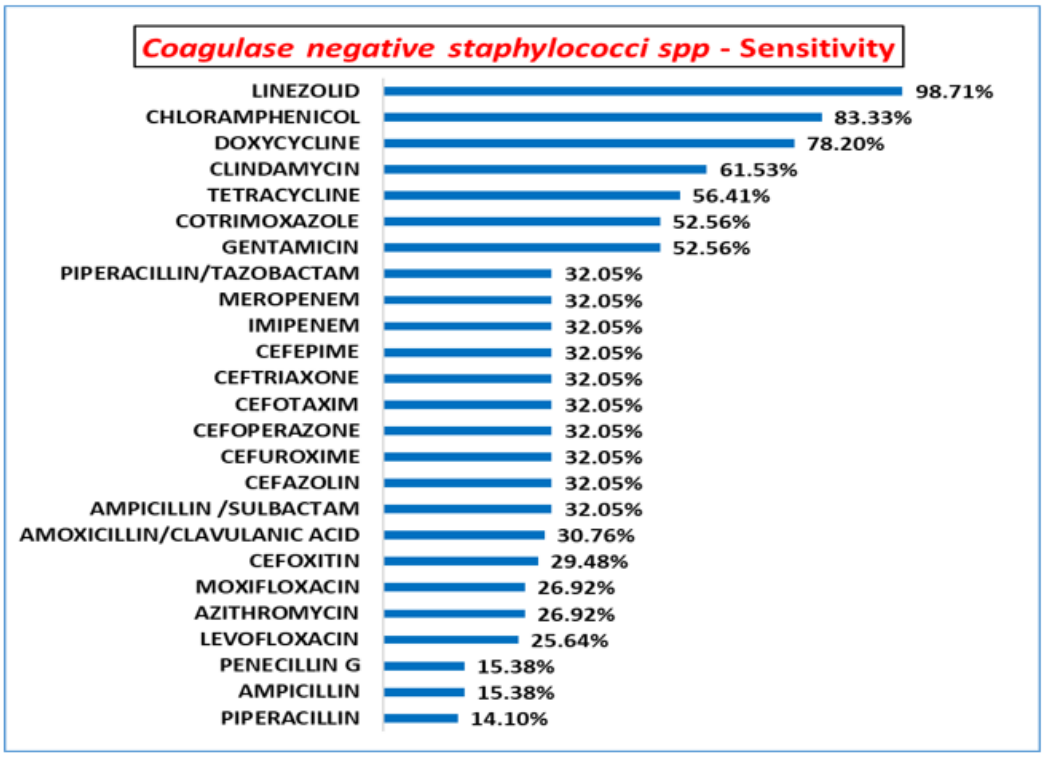


Figure 6

Similar sensitivity patterns were observed for both these isolates showing highest sensitivity for the Linezolid (98%) and decreased sensitivity for the penicillin & cephalosporins. For *S.aureus*, methicillin resistance was seen in 77 (56.20%) isolates out of 137 isolates. In compare to this it was less in *Coagulase*

Negative Staphylococci spp [26 (33.33%) out of 78 isolates]. Linezolid resistance was seen in two *S.aureus*& one *CoNSspp.Enterococcus spp* showed 87% sensitivity to Linezolid, 85% to Vancomycin and <50% for High level gentamicin, Levofloxacin & Tetracycline. Vancomycin resistance (VRE) was seen in 4(10%) *Enterococci* out of 40 isolates. [**Figure.7**]

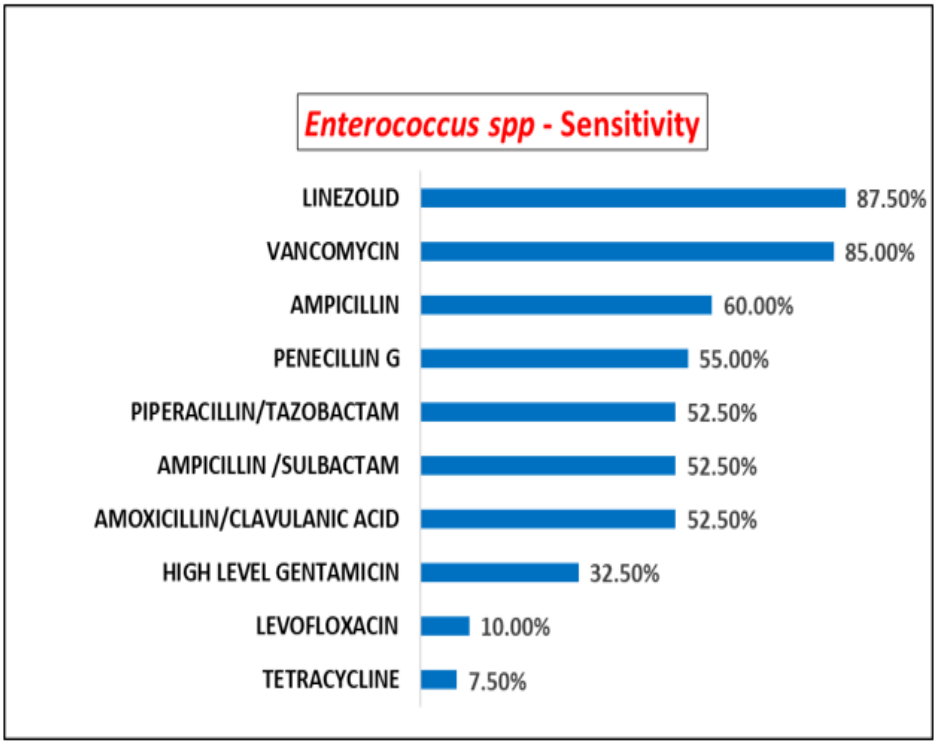


Figure 7

There was increasing resistance observed among both the gram negative &gram positive isolates from various clinical specimens for which they were previously known to be sensitive.

DISCUSSION

There are multiple causes & threats of antimicrobial resistance crisis which is global phenomenon. A survey done by IDSA in 2011 found that nearly 60% of participants had seen pan-resistant & untreatable bacterial infections, subsequently rapid emergence of resistant bacteria was declared as "crisis" by many public health organizations, CDC & WHO.[6]Despite the high prevalence of resistant organisms not enough studies are done by developing countries on its clinical &economical impact.[7] Our study with its limitations is an attempt to understand and formulate institutional antimicrobial usage policy based on its findings. In regard to the major etiological organisms found in our study were similar to different studies done by others. A study done in the current year in Wayanad district of Kerala found predominance of *E.coli*among gram negative bacteria &*S.aureus* in gram positive bacteria.[8]. Another 5 year study published in the Lancet, showing predominance of *E.coli, Klebsiella and Pseudomonas, S.aureus* was reported by Michel *et al.,* in the year 2023.[9] Our study did find similar pattern of isolation of gram negative & gram positive organisms. In our study specimen types distribution showed Pus with maximum culture positive isolates [189(≈30%) gram negative & 159 (≈57%) gram positive major isolates], where as study done in Gwalior India in 2022 found 74.10% gram negative & 20.53% gram positive organisms from Pus sample isolates.[10]*E.coli* was the major isolate type from urine samples (83.3%) in the study done by Belay *etal.*[11] Present study also showed *E.coli* as major isolate type with 72.06%, followed by *Enterococcus spp* with 7.69% from urine samples. Antimicrobial susceptibility of *E.coli* isolates of Belay *et al.,* study showed high resistance to Ampicillin (98.9%) & Cefuroxime (100%), with *Acinetobacter spp* showing almost 100% resistance to the majority of the drugs.[11] Similarly our study showed *E.coli* sensitivity of only 11.34% for Ampicillin &< 50% for cephalosporins except for cefoxitin (52.57%), and <50% sensitivity for the tested drug for *Acinetobacter spp.* Study done by Shekha Nita Mondal *et al.* found *E.coli* &*S.aureus* as major isolate among the various specimen types and they observed higher sensitivity of isolates for Cefepime, Imepenem & Meropenem.[12] In our study major gram negative isolates showed lower <50% sensitivity for Cefepime except *Pseudomonas spp* (62.50%) and Meropenem showed higher sensitivity of > 70% among gram negative isolates but Imepenem showed decreased to Moderate sensitivity. A recent cross sectional study by Nauman Khalid *et al.,* & Saad Alhumaid *et al.,* found high sensitivity of

gram positive isolates for linezolid, vancomycin, chloramphenicol and decreased sensitivity to cephalosporins among gram negative isolates, these findings were similar to our present study findings.[13,14] In retrospective cross-sectional study done by Mengistu Hailemariam *et al.* in Southern Ethiopia and significant sample sized study on Urine isolates done in Iraq also described a similar pattern of gram positive-gram negative isolates from clinical specimens, sensitivity patterns higher rates of multi drug resistant organisms.[15,16] A one year prospective study by Nicholaus P Mnyambwa*et al.*in Tanzania showed high level of resistance many routinely used antimicrobials, with 100% Ampicillin, more than 70% resistance to Amoxyclave, Gentamicin, Tatracycline in *E.coli* isolates where as 66.7% *S.aureus* being MRSA. (methicillin resistant). [17] Our study finding were in correlation with many similar studies done in India and other countries, except minor differences. Routine antimicrobial susceptibility testing and surveillance is required in order to identify newly emerging and remerging resistance mechanisms among the microorganisms and to prepare robust, evidence based antimicrobial usage policy as per the local needs.

CONCLUSION

As antimicrobial resistance mechanisms are becoming the global threat for the health care and its cost comprehensive national and global plans are required to combat it. This retrospective study done at local tertiary care hospital helped to identify the prevailing and developing patterns of antimicrobial resistance among both gram positive and gram-negative organisms from various clinical specimens. There is dire need of timely analysis of changing susceptibility patterns of various organisms in order to prepare and implement evidence based antimicrobial usage policy. As susceptibility of organisms keep changing from time to time and from places to places it is required to analyze local data and frame the antimicrobial stewardship policy based on its outcome.

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Conflict of Interest: None.

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