

Original Research Article

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A study of Bacteriological Profile of Wound Infections with Special Reference to Carbapenemase Producing *Klebsiella* species in a Tertiary Care Hospital

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ABSTRACT

Pyogenic infection refers to an infection that produces pus and is usually characterized by multiple focal inflammations due to microbial growth. The incidence of wound infection varies depending on various factors such as wound condition, microbial load and host defense mechanisms. The predominant wound pathogens are Gram negative bacilli such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, *Proteus spp.* & Gram positive cocci such as *Staphylococcus aureus*, Coagulase negative *Staphylococcus*, *Streptococcus spp.*, *Enterococcus spp.* and anaerobic bacteria such as *Bacteroides*, *Clostridial spp.* *Klebsiella* species acquired resistance to many antibiotic classes including β -lactams, fluoroquinolones, aminoglycosides by different mechanisms results in multidrug resistant strains. This study was performed in the department of Microbiology, in conjunction with the Surgery and Ortho department in a Tertiary care hospital. Microorganisms were identified by using standard methods. Antibiotic susceptibility test was done using modified Kirby Bauer's disc diffusion method on Mueller Hinton Agar as per Clinical and Laboratory Standards Institute Guidelines. Phenotypic identification is done by Combined Disc Test, Double Disc Synergy Test, Modified carbapenem inactivation method (mCIM), EDTA- modified carbapenem inactivation method (eCIM), RapidecCarba NP test. Among those samples 306 organisms showed growth, which are identified and tested for antibiotic susceptibility testing. Among them 128 *Klebsiella* species isolates are grown & from those 11 *klebsiella* isolates showed resistance to Carbapenems and their Carbapenemase production is detected by a set of screening and confirmatory tests. *Klebsiella spp.* has been isolated in higher rates than any other bacteria in our study followed by *E.coli*, *Pseudomonas aeruginosa*. Prevalence of *Klebsiella spp.* is found to be 41.8% in which Carbapenem resistance is found to be 8.5%. Among them about 72.7% are confirmed for Carbapenemase production by mCIM and Rapideccarba NP test and 62.5% isolates are confirmed for MBL production by eCIM tests. This study exemplified that the more number of the *Klebsiella spp.* strains are found to be resistant to numerous antibiotics. To prevent infections due to multidrug resistant *Klebsiella spp.*, a well formed team consisting of eminent treating clinicians, clinical microbiologists and the infection control team's participation with appropriate intercommunication, focussed target action is much need of the hour.

Keywords

Microbial growth,
wound condition,
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Introduction

Pyogenic infection refers to an infection that produces pus and is usually characterized by multiple focal inflammations due to microbial growth (Barer, 2012). It is composed of dead tissue, leukocytes and damaged cells.

The incidence of wound infection varies depending on various factors such as wound condition, microbial load and host defense mechanisms.

Acute pyogenic bacterial infection

Pyogenic means to induce pus. Pus is composed primarily of live and dead neutrophils. The presence of pus generally reflects acute inflammatory processes and activation of the innate immune system. Inflammatory processes can be localized, like the formation of an abscess or spread widely throughout the tissue level. More than a trivial acute suppurative infection is usually accompanied by elevated blood neutrophil counts.

The acute nature of these infections is reflected in their rapid onset. Therefore, the bacteria that cause them generally grow rapidly and form visible colonies within 2 hours of inoculation.

Medical intervention is most effective when done early in the infection, before acquired immunity develops, and, if successful ends the disease. (Barer, 2012)

A wound is a break in the skin and the exposing the subcutaneous tissue areas to all pathogenic microbial organisms providing a damp, warm and enriched environment that is facilitative for colonization and for further proliferation. In developing countries like India, many people die daily of avoidable and curative diseases such as primary and secondary wound infections.

Exogenous wound infection

Exogenous wound infection comprises of those associated with traumatic wound injury or pressure

ulcer, bites of animal or human beings, various degrees of burns, foreign bodies inoculation into the structures of skin or mucous membrane.

Endogenous wounds

Endogenous wounds and abscess may be associated with abscesses of internal organs like appendix, gall bladder, dental structures, bones and articular surfaces, sinuses, etc.,

Surgical site infections (SSIs)

Surgical site infections (SSIs) are the most common nosocomial infections after surgery and are associated with significant morbidity and mortality leading to transfer to intensive care units, prolonged hospital stay and readmissions.

SSIs may be defined as invasion and the multiplication of microorganism in body tissue which may be clinically in apparent or result in local cellular injury because of competitive, toxins, intracellular replication or antigen-antibody response (Gregory *et al.*, 2009)

Surgical site infection (SSIs) is a potential complication associated with any type of surgery. Even though SSIs are among the most avoidable HAIs, they still represent an important burden of patient morbidity and mortality and additional healthcare costs system. SSI is the most studied and the leading HAIs are reported from hospitals in low- and middle-income countries. Most of these processes are health care associated infections are acquired after minimal to maximal invasive procedures, surgical operative procedures, prosthesis placement.

The predominant wound pathogens are. Gram negative bacilli such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Proteus* spp.; Gram positive cocci such as *Staphylococcus aureus*, Coagulase negative *Staphylococcus*, *Streptococcus* spp., *Enterococcus* spp. and anaerobic bacteria such as

Bacteroides, *Clostridia* spp. (Cooper *et al.*, 2003)

Klebsiella species acquired resistance to many antibiotic classes including β lactams, fluoroquinolones, aminoglycosides by different mechanisms results in multidrug resistant (resistant to more than three antibiotic class of drugs) strains. This might be due to as it harbours many virulence factors such as capsule, fimbriae, siderophores and lipopolysaccharides.

Carbapenem is used as first line of therapy for multidrug resistant gram negative pathogens. Resistant to carbapenem spreads through expression of carbapenamase encoded by plasmids or mobile genetic elements which facilitate horizontal transfer of resistance gene between pathogens. Carbapenem resistant Enterobacteriaceae has been reported worldwide.

The aim of this study is to find out common the bacterial isolates and their antibiotic resistance pattern, the incidence of Carbapenem resistance among *Klebsiella* species isolates in pus from wound infections.

The main aim of this study to determine the microbiological profile and its antibiotic susceptibility patterns of bacterial cultures isolated from pus samples and to detect the carbapenamase producing *Klebsiella* species by phenotypic method.

According to the aim of this study objectives were studied that includes to study the bacteriological profile of pus samples from patients admitted in Surgery and Ortho wards. This study was determining the prevalence of *Klebsiella* species causing wound infections. The Antibiotic Susceptibility pattern of bacterial isolates isolated from pus samples was performed. Finally detect the Carbapenem resistance in *Klebsiella* species.

Materials and Methods

Design of the study

This descriptive study was performed in the

department of Microbiology, in conjunction with the Surgery and Ortho department in a Tertiary care hospital.

Ethical Committee Approval

The approval for conducting this study was obtained from the ethical committee of this institution.

Duration of the Study

This study was conducted from 2022 October to 2022 December.

Sample Size

Six Hundred samples.

Study Population

Among patients with abscess and wound infections admitted in the department of Surgery and Ortho of a Tertiary care hospital.

Inclusion criteria

Patients of all age groups attending ortho and surgery OPDs and patients who are admitted in ortho and surgery department wards.

Not taking antibiotic treatment for atleast five days for the current wound infection.

Exclusion criteria

Bed sore patient.

Patients on antibiotic treatment for current episode for more than 2 weeks.

Data Collection

Patient data such as age, gender and duration of illness, mode of occurrence -trauma or insidious onset, duration and details of antibiotic therapy for the current infection, number of days of hospital

stay, comorbid conditions were obtained from patient history and notes. Patients were followed up on outcome.

Sample Collection (Mandell *et al.*, 1995)

Wounds are cleaned using normal saline and scab and decayed materials are removed and swabs are taken from the depth of the wound, Under aseptic precautions pus samples were collected using sterile cotton swabs for open wounds with pus discharge and using sterile syringe with needle for collecting pus from closed abscess.

Sample Transport

Samples are transported to Diagnostic Microbiology Lab without any further delaying.

Sample Processing

First direct Gram staining is done followed by KOH mounting for the identification of bacterial and fungal hyphae respectively. Secondly the swab is for inoculated on Nutrient agar plate, 5% sheep Blood agar plate and also in the MacConkey agar plates. Organisms are identified based on the morphology of the colonies, Gram stain appearance, testing for presence of catalase enzyme, testing for presence of oxidase enzyme, testing of motility by hanging drop method.

The tests are performed with positive control and negative control using ATCC strains. ATCC strains used are: A) *Escherichia coli* (ATCC 25922)

Pseudomonas aeruginosa (ATCC 27853)

Staphylococcus aureus (ATCC 25923)

The following preliminary test were done for isolates grown on solid media.

Colony morphology & Gram staining of culture smear from colony Catalase test, Oxidase test, Motility by hanging drop method.

Antibiotic susceptibility test was done using modified Kirby Bauer's disc diffusion method on Mueller Hinton Agar as per Clinical and Laboratory Standards Institute Guidelines (CLSI, 2020)

Identification of common isolates: A gram negative bacilli that is oxidase negative, catalase positive, ferments glucose and reduces nitrate was identified as member of Enterobacteriaceae family.

A gram negative bacilli that is oxidase positive, catalase positive, does not ferments glucose, was identified as member of non -Enterobacteriaceae family. The following biochemical tests were done to identify the microorganism Biochemical test

Indole; Methyl red; Voges – Proskauer; Citrate; Triple sugar iron(TSI) agar test; Urease; Mannitol Motility Medium; Carbohydrate fermentation - glucose, lactose, sucrose, maltose, mannitol

Gram positive cocci which was catalase positive was identified as *Staphylococcus* spp. Gram positive cocci arranged in clusters which was catalase positive, coagulase positive, produced golden yellow pigmentation, β hemolytic colonies on blood agar plate was identified as *Staphylococcus aureus*.

Gram positive cocci arranged in pairs at an angle to each other which was catalase negative, produced non hemolytic colonies on blood agar, magenta pink colonies on MacConkey agar, hydrolyze bile esculin was identified as *Enterococcus* species.

Gram positive cocci arranged in chains of varying length, catalase negative, does not hydrolyze bile esculin was identified as *Streptococcus* spp.

Antibiotic susceptibility testing was performed for all positive cultures isolates on Mueller Hinton Agar (MHA) by using Kirby - Bauer disc diffusion method as recommended in Clinical and Laboratory

Standards Institute Guidelines (CLSI) M100-2021.(CLSI, 2020; Taneja and Sharma, 2008)

Methods of detection

Screening test for carbapenamase

Phenotypic confirmatory method

Combined Disc Test (Ankur Goyal *et al.*, 2008)

Double Disc Synergy Test (Ya-Min Tsai *et al.*, 2020; Suryavanshi and Lahiri, 2018)

Modified carbapenem inactivation method (mCIM)
EDTA- modified carbapenem inactivation method (eCIM)

RapidecCarbaNP test

Results and Discussion

600 swab samples are collected from patients who attended the department of Surgery and Ortho in a Tertiary care hospital are included in the study. Among those samples 306 organisms showed growth, which are identified and tested for antibiotic susceptibility testing.

Among them 128 *Klebsiella* species isolates are grown & from those 11 *Klebsiella* isolates showed resistance to Carbapenems and their Carbapenamase production is detected by a set of screening and confirmatory tests.

The observations are documented and the results are shown in the following tables and charts:

Out of 600 samples received 70% of samples are from open wounds, 30 % are from closed wounds (abscess). SSI is found to be less than 10 % in case of elective surgeries whereas in case of emergency surgeries the SSI is found to be more than 50 %.

Klebsiellaspp. showed 84.8% sensitivity to Gentamicin, 86.3% to Amikacin, 31% to Cotrimoxazole, 38.3% to Ciprofloxacin, 31% to Amoxyclav, 31% to Cotrimoxazole, 89% to Cefoperazone Sulbactam, 93% to

Piperacillintazobactam, 87.5% to Imipenem.

11 out of 128 *Klebsiellaspp.* isolates demonstrated resistance to Carbapenems in phenotypic screening test and these 8.5% isolates are subjected to phenotypic confirmation for Carbapenamase production.

The bacteriological profile of pyogenic infections may remain the same but the antibiotic susceptibility pattern mostly varies. The occurrence of wound infections relay upon the multiple factors like the type of wound, microbial load and the host defense mechanism. (Nithya Gomatheswari and Jeyamurugan, 2017)

SSIs accounts for 15% of all nosocomial infections and among surgical patients, represents the most common nosocomial infection. Most of the post operative wound infections are hospital acquired, differs from one hospital to another hospital and they pose significant post-operative morbidity, mortality and prolonged in-hospital stay period, leads to direct or indirect enormous increase in the hospitalization cost and to the emergence of novel health issues for the community as stated by Jenna Amatya *et al.*, (2015)

The regular use of antibiotics has resulted in the spread of antibiotic resistance especially within the Gram negative bacteria (Mary Shama *et al.*, 2018). Bacteria do acquire resistance and also have the ability to transfer the resistance among each other. Before such multidrug resistant organisms were predominantly seen in immunocompromised patients but now they are becoming common in normal healthy persons.

These multi-drug resistant infections worsen the newly emerging infectious diseases. Post operative wound infections have always been a happening in human life. Sepsis in old or modern surgery procedures continues to be a very significant problem for the surgeons across the globe.

Patients who underwent with some surgical

procedures, represent a significant burden accounting to morbidity and mortality leading to increasing cost to the health care services across the globe (Anderson *et al.*, 2014 and Chia-Hsiao Kuo *et al.*, 2004). Out of the culture positive patients, majority of the patients were found between the age group 41 to 60 years and the least cases above 80 years of age. It was found that surgical site infection was mostly prevalent in the working age group 41-60. People in this age group of 20-40 years being active in all works explaining why most wound infections occur in this group by studies done by Chia-Hsiao *et al.*, (2004). 200 patients were found to be diabetic in our study. The present study supports the results of Swati Duggal *et al.*, (2015) that increased blood glucose levels is a significant preoperative risk factor for the development of surgical site infections. Certain host factors influence the chances of post-operative wound infection such as advanced age, poor nutrition, increased body mass index, operating site and comorbid conditions like diabetes mellitus, Tuberculosis, Anemia, Tumors & HIV, etc.

In this study, both Gram positive and Gram negative pathogens were isolated from the pus samples. The predominant bacterial pathogens isolated were Gram negative bacteria. This was same by the study done by Shama *et al.*, (2018); Mita D Wadekar *et al.*, (2000); Rugira Trojan *et al.*, (2016) which showed the predominantly grown pathogen as Gram negative bacteria.

This is also supported by the results of Premchandran *et al.*, found that Gram-negative bacteria namely *Klebsiella*, *E.coli* and *Pseudomonas* isolates were predominant pathogens along with Gram-positive bacteria. But this is against other studies which says 69% for GPC & 29% for GNB by Surucuoglu *et al.*, (2005) and by other the studies by Tellis *et al.*, (2013); Anil Rajput *et al.*, (2012); Artikapil *et al.*, (2005) where Gram positive bacteria were the predominant ones being isolated.

There are numerous studies with similar results and there is a moving trend towards the Gram-negative

organisms becoming highly prevalent in wound infections. However, some studies still reveal that *S.aureus* is the predominant bacterial pathogen followed by Gram-negative bacterial pathogens. *Klebsiella* was isolated in higher rates than any other bacteria in our study with isolation rate of 38%, which is similar to other studies conducted by Anderson *et al.*, (2014).

In the present study *Klebsiella spp.* showed 93% sensitivity to Piperacillin/tazobactam, 87.5% to Imipenem, 89% to Cefoperazone Sulbactam, 86.3% to Amikacin, 84.8% sensitivity to Gentamicin, 31% to Amoxycylav, 31% to Cotrimoxazole, 38.3% to Ciprofloxacin. In contrast to our study, in the study by Jonathan osariemenisibor *et al.*, (2008) sensitivity pattern of *Klebsiella pneumonia* was 100% to Gentamicin, 50% to Ciprofloxacin. In contrast to this finding, sensitivity pattern of *Klebsiella pneumonia* was found to be 100% sensitive to Imipenem, 47.37% to Gentamicin, 37.62% to Ciprofloxacin (Misra Yogesh Chander *et al.*, 2000).

Most of Gram negative isolates in our study were susceptible to piperacillin/tazobactam 92.7% and Carbapenem 85.4% and Cefoperazone Sulbactam 94.05% which is similar to study done by Artikapil *et al.*, (2005) which showed maximum susceptibility to these antibiotics.

Among the Gram positive pathogens isolated in our study *Staphylococcus aureus* – the most commonly grown bacteria followed by CoNS and Enterococci which is similar to the study conducted by Artikapil *et al.*, (2005) *Staphylococcus aureus* is one of the normal flora of the skin commonly associated with wound infection. Many of the Gram positive bacterial isolates were found to be sensitive to vancomycin (100 %) and linezolid (92.5%) which is similar to the studies done by Shittu *et al.*, (2002). The difference in antibiotic sensitivity pattern of these bacterial isolates was probably due to the difference in the condition of the patient, the antibiotic used for empirical treatment, and also this antibiotic sensitivity patterns might change from

place to place and time to time. So hospital antibiograms are generally employed to help guide empirical antibacterial treatment and are crucial

component of finding and monitoring the evolving trends in antibiotic resistance.

Table.1 Age Wise Distribution

0-20 years of Age	13
21-40 years of Age	72
41-60 years of Age	124
61-80 years of Age	94
>80 years of Age	6
TOTAL PATIENTS	306

Table.2 Types of Wounds among the Study Population

(N=600)

Type of Wound	Open Wound	Close Wound
Percentage	70%	30%

Table.3 Growth on Culture Distribution

Culture Positive	Percentage	Culture Negative	Percentage
306	51.5%	294	48.5%

Table.4 Monomicrobial and Polymicrobial Growth Pattern

(n=306)

Monomicrobial Growth	Polymicrobial Growth	Monomicrobial Percentage	Polymicrobial Percentage
306	4	98.7%	1.3%

Table.5 Bacterial and Fungal Isolates Distribution

(N= 306)

Bacterial Growth		Fungal growth	Bacterial percentage	Fungal percentage
306	2		99.35%	0.65%

Table.6 Gram Positive Cocci Vs Gram Negative Bacilli

(N=306)

Gram Positive Cocci	Gram Negative Bacilli
71(23.3%)	235 (76.7%)

Table.7 Microbiological Profile of Pus

Organism grown	Number of Isolates (n=306)	Percentage of the growth
<i>Klebsiella spp.</i>	128	41.8%
<i>Escherichia coli</i>	37	12.7%
<i>Pseudomonas aeruginosa</i>	33	10.7%
<i>Acinetobacter baumannii</i>	26	8.4%
<i>Staphylococcus aureus</i>	25	8.16%
CONS	24	7.8%
<i>Proteus spp.</i>	20	6.5%
<i>Enterococcus spp.</i>	6	1.9%
<i>Citrobacter spp.</i>	4	1.3%
<i>Enterobacter spp.</i>	1	0.65%
<i>Candida spp.</i>	2	1.2%

Table.8 *Klebsiella* Spp. Isolates - Antibiotic Susceptibility Pattern

(n= 128)

Antibiotic used	Sensitivity	Resistant
Gentamicin	108(84.8%)	20(15.6%)
Amikacin	110(86.3%)	18(14%)
Cotrimoxazole	41(31%)	87(68%)
Ciprofloxacin	49(38.3%)	81(63%)
Cefotaxime	65(50.9%)	63(49%)
Amoxyclav	40(31%)	88(69%)
Cefoperazone Sulbactam	114(89%)	14(11%)
Piperacillintazobactam	119(93%)	9(7%)
Imipenem	112(87.5%)	16(12%)

Table.9 Overall Susceptibility Pattern Of Gram Negative Bacilli

Antibiotic	Sensitive %	Resistance%
Gentamicin	83%	17%
Amikacin	83%	17%
Cotrimoxazole	30%	70%
Ciprofloxacin	39.5%	60.5%
Cefotaxime	47.5%	42.5%
Ceftazidime	21%	79%
Amoxyclav	24%	76%
Cefoperazone Sulbactam	78%	22%
Piperacillintazobactam	92%	8%
Imipenem	94%	6%

Table.10 Percentage of MRSA and MSSA

(n=49)

MRSA %	MSSA
8.3 %	91.7 %

Table.11 Detection of Carbapenem Resistance among *Klebsiella* Spp. Isolates - Phenotypic Screening Test (n=128)

Total Samples	Carbapenemase Screening test Positive- numbers	Carbapenemase Screening test Positive- percentage
128 (n)	11	8.5%

Table.12 Detection of Carbapenem Resistance Among *Klebsiella* Spp. Isolates - Phenotypic Confirmatory Test – mCIM

(n=11)

Total Samples	Carbapenemase Confirmatory test Positive- numbers by mCIM/ Rapidec Carba NP	Carbapenemase Confirmatory test positive- percentage by mCIM / Rapidec Carba NP
11(n)	8	72.7 %

Table.13 Detection of Carbapenem Resistance Among *Klebsiella* Spp. Isolates - Phenotypic Confirmatory Test

Total Samples	Metallobeta Lactamase Confirmatory test Positive- numbers by eCIM	Metallobeta Lactamase Confirmatory test Positive- percentage by eCIM
8 (n)	5	62.5%

Chart.1 Culture Growth Distribution (n=600)

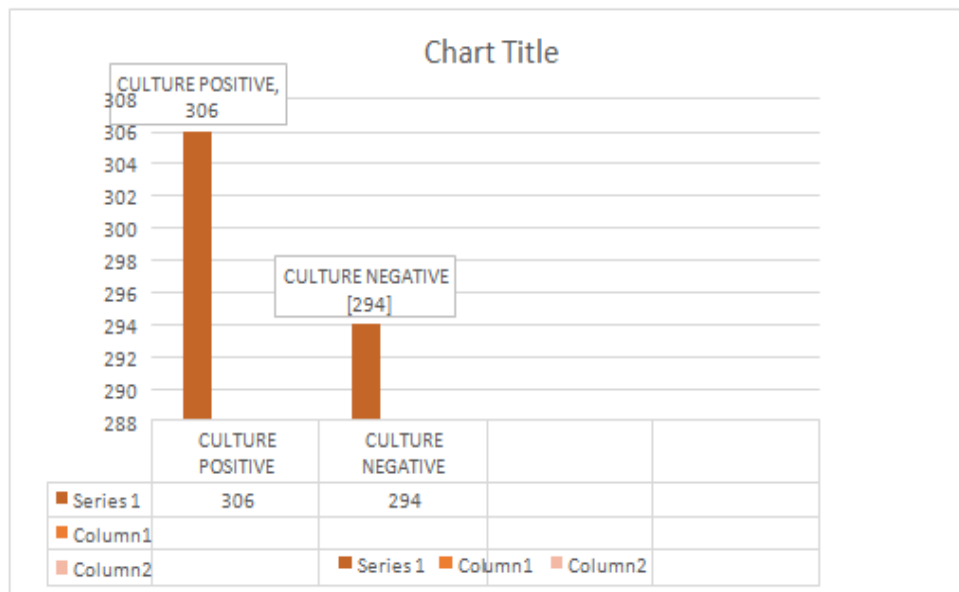


Chart.2 Microbiological Profile of Pus

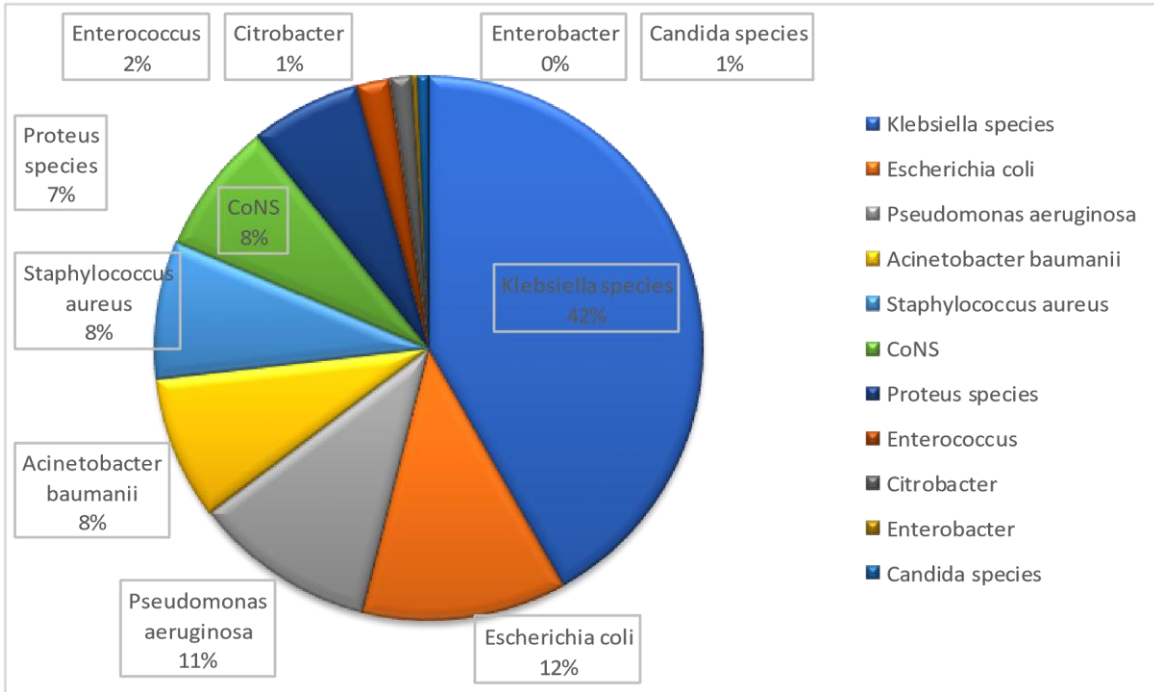


Chart.3 *Klebsiella* Spp. Isolates Antibiotic Susceptibility Pattern (n= 128)

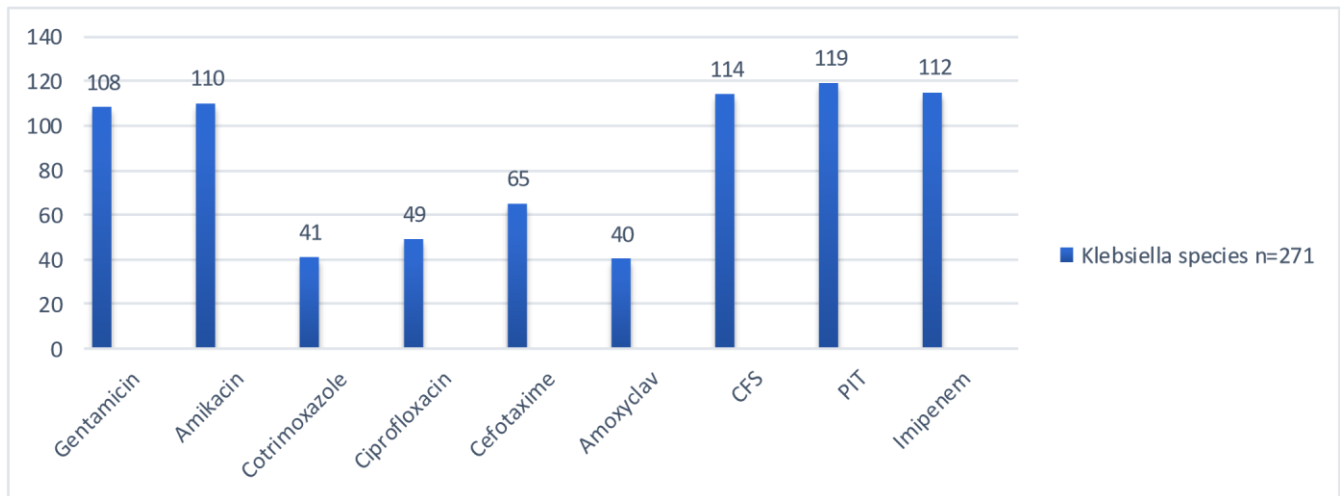
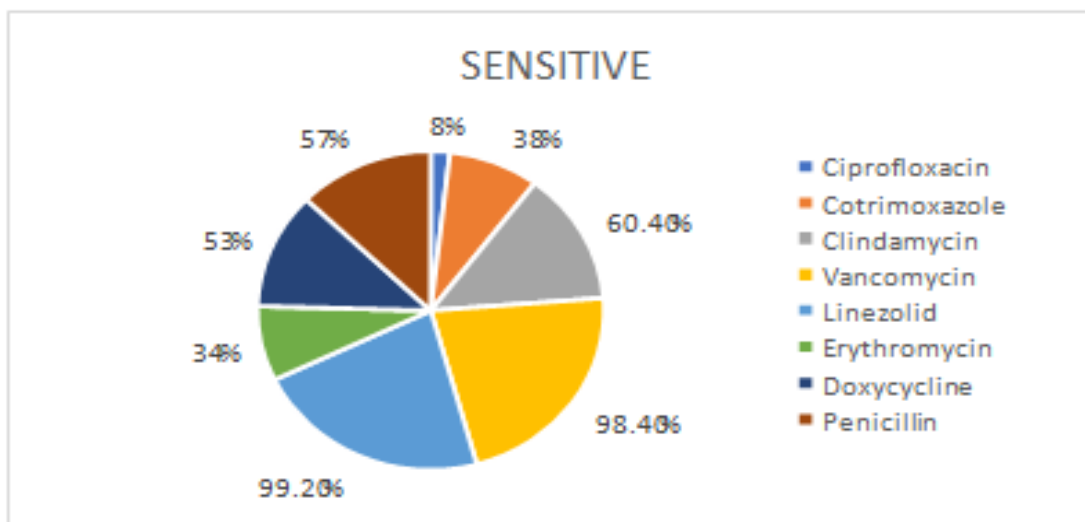


Chart.4 Overall Resistance of Gram Positive Cocci



As antibiotic resistance among Gram positive and Gram negative organisms is increasing, it has now become compulsory to choose antibiotics wisely and to deliver those at correct dosage and duration. Our study also portrayed the existence of high drug resistance to many classes of antibiotics from the clinical sample.

In this study 76.7% are found to be Gram negative bacilli and 23.3% are Gram positive cocci. *Klebsiella* spp. has been isolated in higher rates than any other bacteria in our study followed by *E.coli*, *Pseudomonas aeruginosa*. 8.3 % are MRSA whereas 35.5% isolates are MRCoNS. Majority of the Gram negative bacteria are sensitive to Gentamicin, Amikacin, *Piperacillin tazobactam*, Imipenem with sensitivity percentage as 83%, 83%, 92%, 94% respectively. The overall sensitivity to Carbapenems among the Gram negative organisms was found to be above 90 %. The maximum resistance shown by majority of Gram negative bacilli to Ceftazidime and Amoxyclav. Prevalence of *Klebsiella* spp. is found to be 41.8% in which Carbapenem resistance *Klebsiella* spp isolates are found to be 8.5%. Among them about 72.7% are confirmed for Carbapenemase production by mCIM and Rapideccarba NP test and 62.5% isolates are

confirmed for MBL production by eCIM tests. This study exemplified that the more number of the *Klebsiella* spp. strains are found to be resistant to numerous antibiotics. The inflated antibiotic resistance rates among *Klebsiella* spp. are very startling and can be responsible for more serious infections, leading to a quandary situation. To prevent infections due to multidrug resistant *Klebsiella* spp, a well formed team consisting of eminent treating clinicians, clinical microbiologists and the infection control team's participation with appropriate intercommunication, focussed target action is much need of the hour. Carbapenem is considered as a valuable treatment option for patients suffering from multi resistant *Klebsiella* spp. Infections.

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