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Study of Aerobic Bacteria Isolated from Pus Samples in Tertiary Care Hospital

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Aerobic bacteria, Antibiotic, Isolates, Tertiary care hospital.

ABSTRACT
The study aimed to extract and identify aerobic bacteria from pus samples and investigate antibiograms at a rural tertiary care hospital, providing guidance for empirical therapy.

Material & Methods: The study processed pus samples in the Microbiology laboratory, conducted from November 2020 to October 2021 adhering to established standards. Antimicrobial sensitivity testing employed Kirby Bauer's disc diffusion method, following CLSI recommendations.

Results: Prevalent bacteria included *Staphylococcus aureus* (36.79%), *Klebsiella pneumoniae* (19.49%), and *Escherichia coli* (9.09%). Gram-negative bacteria dominated (77%), with common use of Cefoperazone salbactum, Imipenam, Meropenam. Rising concerns involve Methicillin- resistant Staphylococcus aureus (MRSA) and Vancomycin-resistant Enterococcus (VRE), underscoring the importance of antibiotic sensitivity testing.

Conclusion: The study identifies prevalent bacterial isolates, emphasizing Gram-negative dominance and effective antibiotics. Concerns about MRSA and VRE prevalence highlight the need for targeted interventions and antibiotic susceptibility testing, shaping strategies for vulnerable demographics.

Introduction:

Microbial pathogens may cause infections in human skin and soft tissues after trauma, burns, surgical operations, and other similar events, leading to the formation of pus. The findings of this study might serve as a valuable reference for empirical treatment. [1] Pus is a very identifiable indicator of an illness. Pus, usually referred to as such, is the viscous, white, and odorless exudate that is produced as a result of pyogenic bacterial infections. [2] Antimicrobial resistance (AMR), particularly in Grams negative bacteria, has become a prominent worldwide public health concern due to the limited availability of effective treatment alternatives. [3]. Infections caused by resistant organisms have been shown to be associated with elevated death rates and heightened economic burdens. The presence of pus is considered to be an indication of suppurative infections produced by pyogenic bacteria. [4] A wound refers to a disruption in the integrity of the skin resulting from an injury.

The growth and multiplication of bacteria inside a wound have the potential to result in the development of a wound infection. [5] The majority of the latter instances are acquired inside hospital environment, often as a result of an invasive medical treatment or surgical intervention. Pus is a viscous substance that ranges in colour from white to yellow. [6] As a result, these infections have a substantial impact on the health and well-being of patients, leading to increased rates of illness and death. Pyogenic infections may manifest as either exogenous or endogenous in nature. Exogenous infections often arise in conjunction with severe injuries, burns, and similar conditions, whereas endogenous infections and abscesses are commonly linked to appendicitis, cholecystitis, and related ailments. [7]

The occurrence of anaerobic bacteremia is infrequent, with a range of 0.5% to 9% of all positive blood cultures seen within hospital settings. [8] The majority of the endogenous microbial flora consists of anaerobic bacteria, which have the potential to produce bacteremia [9] analyzed aerobic bacteriological composition and antibiotic resistance patterns in pus samples, highlighting the need for prudent antibiotic use to limit drug-resistant bacteria's proliferation [10] understudied the antibiotic susceptibility patterns in wound samples, addressing antimicrobial resistance, enhancing patient satisfaction, and reducing hospitalization duration in managing pyogenic infections [11] emphasizes the importance of studying pyogenic wound infections and their antimicrobial susceptibility patterns to address drug resistance in bacterial isolates and ensure proper antibiotic administration. [12] Bacterial resistance to antibiotics, providing insights for healthcare professionals to make informed decisions on treatment, considering geographical disparities and multidrug-resistant bacteria.

Materials and method

Pus samples received in two sterile swabs were sent immediately for processing to the bacteriology section, one swab was used for direct Gram's staining & other swab for culture. Samples were inoculated on to Blood agar, MacConkey agar, Nutrient agar media and the plates were incubated at 37^0c for 24 to 48 hrs. Under aerobic condition. Identification of the isolates from culture positive samples were done using standard microbiology laboratory operating procedures like Gram's stain, motility testing and biochemical reactions like catalase, coagulase, indole, methyl red, voges-proskauer, citrate, urease, phenyl pyruvic acid test & oxidase test.

Criteria for antimicrobial sensitivity testing are carried out as per Clinical laboratory standard institute (CLSI). Antibiotic sensitivity testing is done on Muller Hinton Agar by Kirby-Bauer's disc diffusion method. Commercially available discs (Hi-media) are used.

Methicillin-Resistant Staphylococcus aureus (MRSA) and Vancomycin-Resistant Enterococci (VRE)

Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant *Enterococci* (VRE) are notable antibiotic-resistant bacteria implicated in healthcare-associated infections. MRSE commonly causes infections in individuals those with medical devices. Identification involves Gram staining, culture, and molecular methods. Vancomycin-resistant strains of *Enterococci*, namely *Enterococcus faecalis* and *Enterococcus faecium*, constitute VRE. VRE infections, often acquired in healthcare settings, pose challenges as they resist Vancomycin, a key antibiotic. Laboratory methods, including culture and susceptibility testing, help identify and guide treatment. Both MRSA and VRE underscore the importance of stringent infection control measures in healthcare to curb their spread and limit the development of antibiotic resistance.

Results:

Table 1 Antibiotic Sensitivity of Grams positive isolates in pus cultures in our study

Organism **Antibiotics**	***Staphylococcus aureus* n=170**	***Coagulase negative Staphylococcus* n=30**	***Streptococcus species* n=6**	***Enterococcus* n=17**
Ampicillin	-	-	-	50%
Cefoxitin	56%	56%	-	-
Ciprofloxacin	11.7%	40%	33%	87%
Clindamycin	81%	75%	100%	50%
Cotrimoxazole	45%	43%	100%	52%
Erythromycin	23%	25%	100%	50%
Gentamicin	-	53%	-	-
Linezolid	98%	100%	100%	90%
Teicoplanin	-		100%	100%
Tetracycline	73%	33%	100%	33%
Vancomycin	100%	100%	100%	82%
Doxycycline	91%	90%	100%	-
Chlorampenicol	63%	25%	-	-
Penicillin	5%	0	100%	7%

The antibiotic susceptibility of isolates of *Enterococcus*, *Streptococcus* species, *Coagulase- negative Staphylococci*, and *Staphylococcus aureus* from pus sample cultures is shown in the above table. Vancomycin had the greatest effectiveness of the four tested antibiotics, with sensitivity rates of 100%, 100%, 100%, and 82% against the respective species, based on the data shown in the table. Linezolid has been shown

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to be very effective against each of the four infections, with sensitivity rates of 98%, 100%, 100%, and 90% for each distinct organism. Teicoplanin (which has a 100% success rate against *Streptococcus* species and *Enterococcus*), Clindamycin (which has a success rate ranging from 81% to 100%), and Ciprofloxacin (which has a success rate ranging from 33% to 87%), are other antibiotics that demonstrate wide activity against all four infections. However, there is some variation in the level of sensitivity of certain organisms to antibiotics.

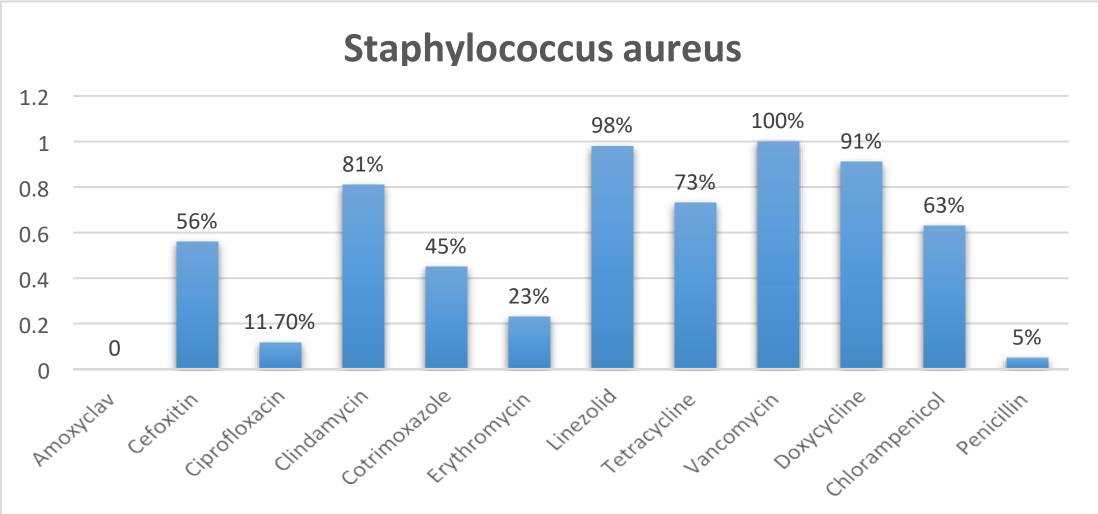

Figure 1 Staphylococcus aureus

The bar graph above illustrates the percentage of *Staphylococcus aureus* isolates susceptible to antibiotics. The y-axis shows the proportion of isolates sensitive to each antibiotic, while the x-axis lists the antibiotics. Most *Staphylococcus aureus* isolates are sensitive to Vancomycin (98%), Linezolid (91%), and Ciprofloxacin (73%). Fewer isolates are sensitive to Erythromycin (45%), Clindamycin (23%), and Tetracycline (11.7%). Since just 8% of isolates are sensitive to penicillin, it is ineffective against Staphylococcus aureus. Overall, the bar graph shows a growing trend in *Staphylococcus aureus* antibiotic resistance. The above problem hinders public health by making it difficult to control infections caused by these germs.

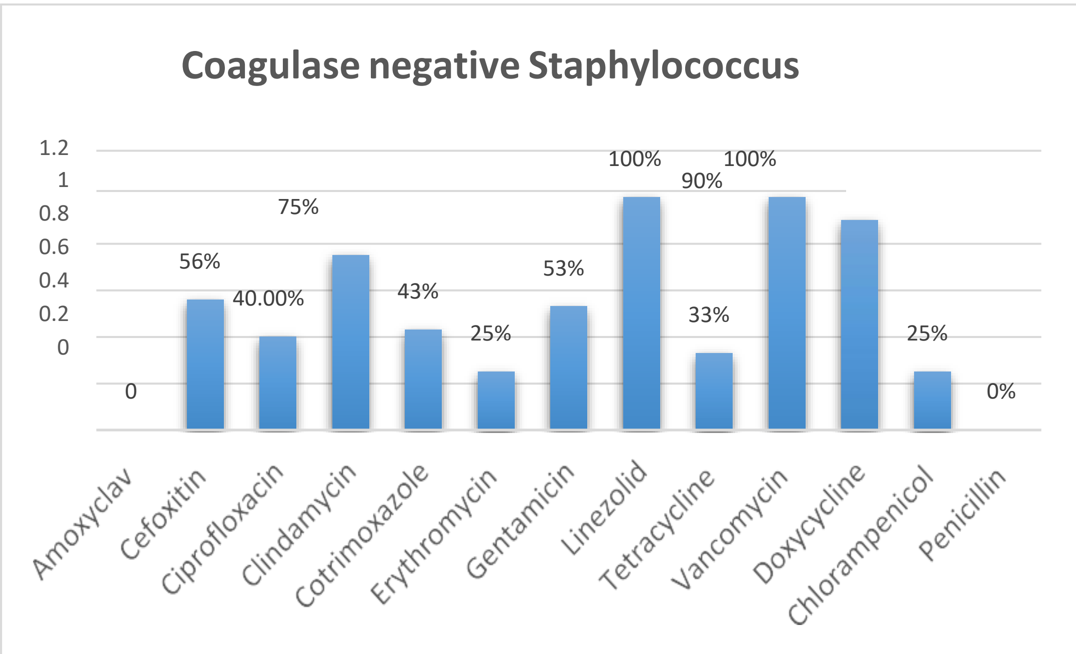

Figure 2 Coagulase negative staphylococcus

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The graph above shows the antibiotic susceptibility of pus-cultured coagulase-negative staphylococci isolates. Most *Coagulase-negative Staphylococcus* isolates are susceptible to Vancomycin (100%), Linezolid (90%), and Gentamicin (75%). Fewer isolates are sensitive to Amoxyclav (56%), Cefoxitin (43%), and Ciprofloxacin (25%). Erythromycin, clindamycin, and Cotrimoxazole had the lowest effectiveness rates against *Coagulase-Negative Staphylococci*, 10%, 9%, and 8%, respectively. This suggests that evaluating *Coagulase-negative Staphylococcus* isolates for antibiotic sensitivity before treatment is crucial.

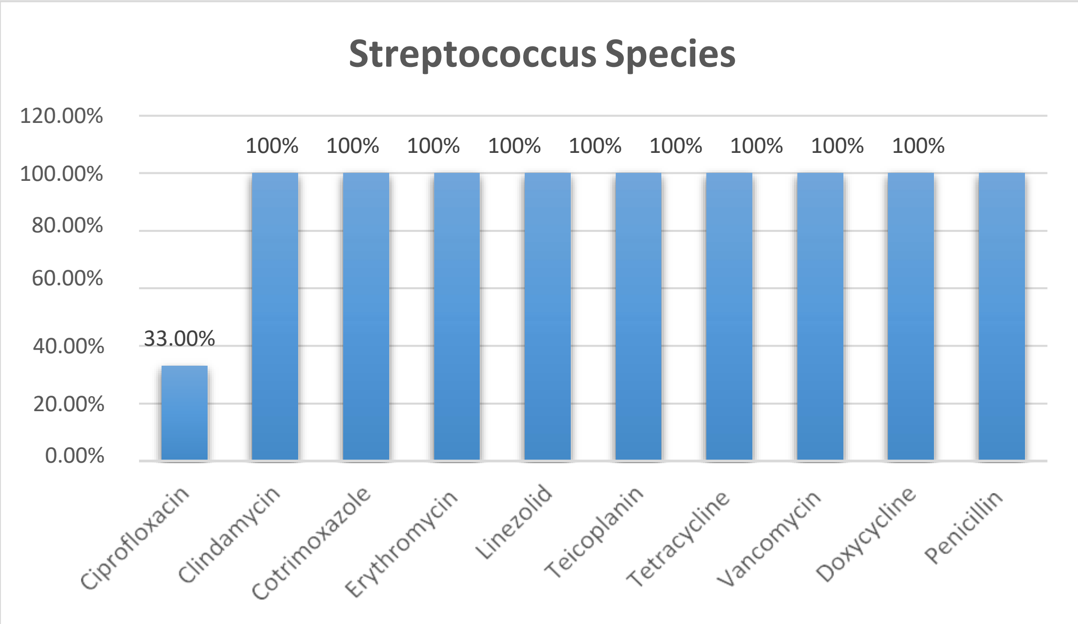

Figure 3 Streptococcus Species

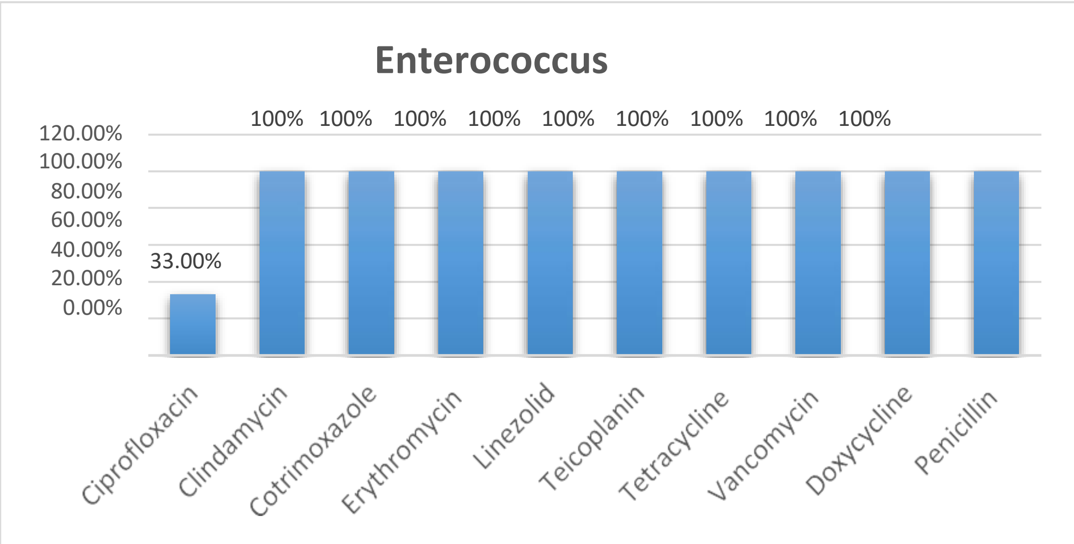

Figure 4 Enterococcus

This graph demonstrates the antibiotic susceptibility of *Enterococcus* Grams-positive isolates from pus cultures in a research study. The plot shows that most Grams-positive isolates are susceptible to Clindamycin (100%), Cotrimoxazole (100%), Erythromycin (100%), Linezolid(100%), Teicoplanin (100%), Tetracycline (100%), Vancomycin (100%), Doxycycline (100%), Penicillin (100%) and Ciprofloxacin (33%). Grams-positive isolates must be tested for antibiotic susceptibility before treatment.

Table 2 Distribution of Pus samples in various age groups in our study

Age group	Number of samples	Percentages
0-10	22	4.76%
11-20	26	5.62%
21-30	63	13.64%
31-40	77	16.66%
41-50	87	18.84%
51-60	110	23.8%
61-70	52	11.26%
>70	25	5.42%

The above table shows the distribution of pus samples in various age groups, our study shows 51- 60 age group were more prone for infection followed by 41-50 years

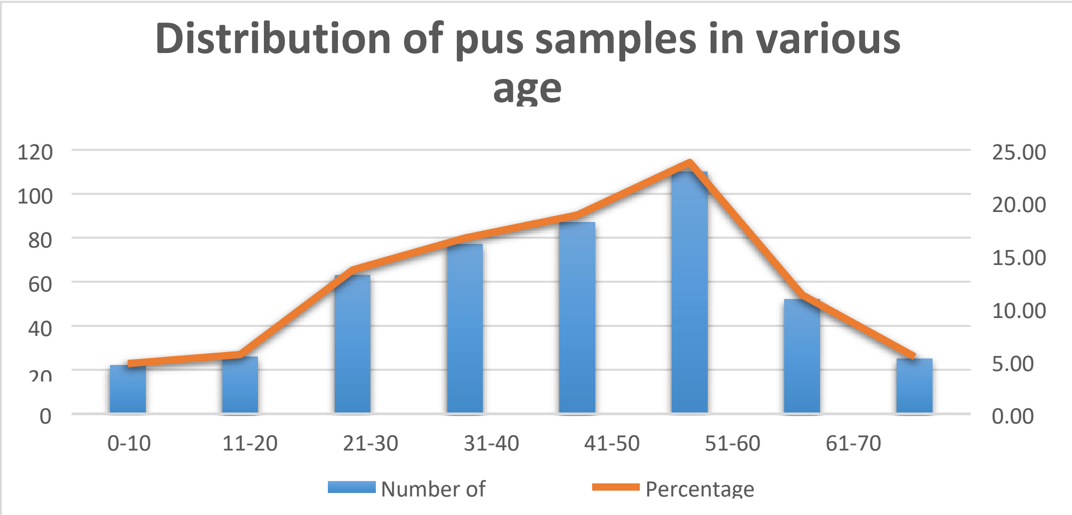

Figure 5 Distribution of Pus samples in various age groups

Pus sample distribution by age is seen in the graph above. The graph shows that pus samples increase with age, peaking in the 51–60 age range and dropping somewhat in the elderly. The 51-60 age group generates the most fluid samples (23.8%), followed by 41-50 (18.84%) and 31-40 (16.66%). Children aged 0–10 had the fewest pitfall samples (4.76%). This figure shows that infection risk increases with age till 60. This may be due to age-related immunological decline and the frequency of underlying medical disorders that enhance infection risk. Additionally, it shows that sputum sample proportions vary greatly by age. This suggests that infection risk varies by population.

Table 3 Prevalence of isolates in pus cultures in our study

Organism isolated	Number of isolates	Percentage
Escherichia coli	42	9.09 %
Staphylococcus aureus	170	36.79 %
Klebsiella	90	19.49 %
Coagulase negative Staphylococcus	30	6.49 %
Proteus	22	4.76 %
Pseudomonas	57	12.34 %
Streptococcus pyogenic	06	1.30 %
Enterococcus	16	3.46 %
Acinetobacter	21	4.54 %

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Enterobacter	01	0.22 %
Edwardsiella	01	0.22 %
Citrobacter	02	0.44 %
NFGNB	04	0.86 %

The table above displays pus culture bacterial isolate distribution. The most prevalent bacterial isolate is *Staphylococcus aureus* (170, 36.79%), followed by *Klebsiella* (90, 19.49%) and *Escherichia coli* (42, 9.09%). *Pseudomonas* (57, 12.34%), *Coagulase-negative Staphylococcus* (30, 6.49%), and *Enterococcus* (16, 3.46%) are rarer. Research shows that *Staphylococcus aureus* causes most pus-culture-requiring bacterial infections. *Staphylococcus aureus* is a frequent skin inhabitant and may infiltrate via wounds or scrapes. *Staphylococcus aureus* may infect skin, wounds, and bones.

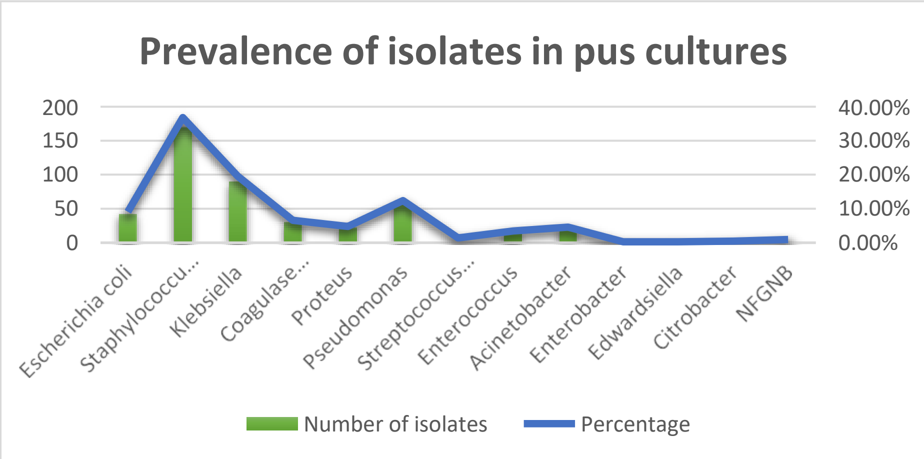

Figure 6 Prevalence of isolates in pus cultures

The graph shows pus culture isolation predominance. At 40% of cases, E. coli is the most common isolate. Following is *Staphylococcus aureus*, 30% of isolates. *Klebsiella* and *Coagulase-negative Staphylococci* account for 20% and 10% of prevalence. While Gram-positive bacteria are still common, Gram-negative bacteria such *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Edwardsville*, and *Citrobacter* dominate. This data aids empirical pus infection treatment choices.

Table 4 Antibiotic sensitivity of Grams negative isolates in pus cultures in our study

Organism / Antibiotics	E. coli n=42	Klebsiel la n=90	Proteus n=23	Pseudo monas n=57	Acinet obacte r n=21	Entero bacter n=1	Edwards ville n=1	Citrobacte r n=2	NFG NB n=4
Ampicillin	-	-	50%	-	-	-	-	-	-
Amikacin	71%	84%	100%	89%	57%	-	100%	100	50%
Cefazolin	16%	19%	58%	-	-	-	-	-	-
Cefipime	20%	56%	90%	40%	-	-	-	-	50%
Cefoperazon e/salbactum	100%	100%	100%	100%	100%	100%	100%	100%	100%
Cefotaxime	25%	24%	100%		48%	-	-	-	-
Ceftazidime	27%	48%	95%	64%	29%	100%	100%	50%	100%
Ceftriaxone	28%	17%	100%		-	-	-	-	-
Ciprofloxacin	26%	20%	100%	70%	48%	-	-	-	-
Colistin	100%	100%	-	-	100%	-	-	-	100%
Cotrimoxazole	-	40%	100%	-	48%	-	-	-	-
Gentamicin	42%	74%	84%	90%	45%	100%	100%	50%	-
Imipenem	94%	86%	95%	100%	91%	100%	100%	100%	100%
Meropenem	93%	81%	100%	100%	100%	100%	100%	50%	100%
Nalidixic Acid	--	--	--	-	-	-	-	-	-
Piperacillin/ Tazobactam	41%	60%	80%	67%	86%	-	-	-	-
Tobramycin	55%	86%	73%	89%	38%	-	-	100%	-

The table shows the antibiotic susceptibility of 240 isolates from eleven bacterial species. This table shows the percentage of isolates susceptible to each antibiotic. For instance, *Escherichia coli* isolates were 94% susceptible to Imipenem but just 16% to cefazolin.

Table 5 Gender Distribution

Gender distribution	N=462	Percentage
Male	331	71.65 %
Female	131	28.35 %

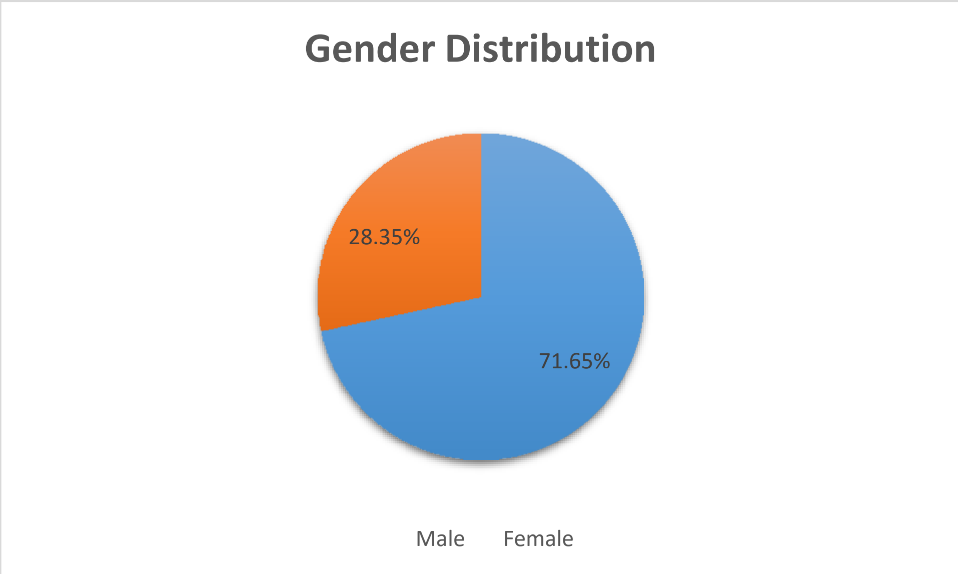

Figure 7 Gender Distribution

The pie chart illustrates the distribution of individuals by gender within a sample size of 462. 71.55% of the population is male, whereas 28.35% is Female, as indicated by the pie chart. This indicates that the population consists of 331 males and 131 females.

Discussion

The current investigation demonstrated that *S. aureus* is the predominant pathogen found in pus samples, which is consistent with the findings of Tiwari P. et al [13] and Lee C.Y. et al [14]. However, Agnihotri N et al [15] identified *Pseudomonas spp* as the second most prevalent pathogen. In our investigation, *Pseudomonas* and *Escherichia coli* spp. were the predominant Gram Negative Bacilli (GNB) found in the pus samples. The aerobic growth in pus culture has been strongly supported by investigations conducted by Ghosh A et al [16] and Zubair M. et al [17], indicating the significant dominance of GNB. In addition, a study conducted by Basu S. et al [18] found that *Pseudomonas* and *Escherichia coli* spp. were the predominant pathogens in wound infections, with *Pseudomonas* being more prevalent than E. coli. A study conducted in North India [19] found that 71.82% of bacteria from wound infections were Gram-negative, whereas 28.18% were Gram-positive. Additionally, 63.2% of these bacteria were identified as multidrug-resistant (MDR). In a separate investigation carried out in Nepal [20], Gram-negative bacteria were the primary microorganisms identified in wound infections.

Our study shows a thorough examination of pus cultures obtained from a wide range of patients, elucidating the susceptibility of different bacterial isolates to antibiotics, age-related patterns in infection rates, prevalence of distinct bacterial species, and gender distribution within the population under investigation.

The antibiotic sensitivity results provide significant information regarding the effectiveness of various antibiotics against Gram-positive isolates, such as *Staphylococcus aureus, Coagulase- negative Staphylococcus, Streptococcus species, and Enterococcus*. Vancomycin demonstrates exceptional efficacy against all Gram-positive organisms, exhibiting a sensitivity rate of 100%. Linezolid exhibits notable efficacy, ranging from 90% to 100%, against several species. Clindamycin, Teicoplanin, and Ciprofloxacin have a wide range of effectiveness, highlighting their promise in treating infections caused by these Gram-positive isolates. Nevertheless, disparities in susceptibility among various species underscore the significance of employing a focused approach in antibiotic choice, guided by microbial identification.

The bar graph illustrates the susceptibility of Staphylococcus aureus to different antibiotics, highlighting the high effectiveness of Vancomycin (98%), Linezolid (91%), and Ciprofloxacin (73%). The data demonstrates an increasing pattern of antibiotic resistance, emphasizing the necessity for customized treatment strategies and the significance of conducting susceptibility tests prior to commencing therapy.

The data pertaining to *Coagulase-negative Staphylococcus*, *Streptococcus* species, and *Enterococcus* provide additional clarity on the susceptibility of antibiotics, highlighting the variations in reactions among Gram-positive isolates. It is repeatedly emphasised to do pre- treatment antibiotic sensitivity testing in order to maximize therapeutic outcomes.

An analysis of pus samples across various age groups yields useful demographic insights. An examination of age groups indicates a rising pattern in pus samples as age increases, reaching its highest point in the 51-60 age group. This insight is essential for comprehending the fluctuations in infection risk that are associated with the reduction in immune function due to ageing and the presence of underlying medical disorders. The graphical representation effectively demonstrates these data, showing a fourfold rise in pus culture samples among individuals aged 51–60 compared to those aged 0–10. Our study revealed that *Staphylococcus aureus* is the most prevalent, accounting for 36.79% of the samples, followed by *Klebsiella* at 19.49% and *Escherichia coli* at 9.09%. This information is crucial for physicians as it offers valuable insights into the infections that are frequently encountered and helps in making informed decisions for empirical treatment.

The antibiotic susceptibility of Gram-negative isolates, including as *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Edward-sville*, *Citrobacter*, and NFGNB, provides a comprehensive assessment of the efficacy of several antibiotics against these pathogens. The range of susceptibility highlights the significance of selecting antibiotics specifically tailored to the microbial identity. Finally, the examination of gender distribution demonstrates a greater incidence of pus cultures in males (71.65%) as opposed to females (28.35%). This information necessitates additional examination of potential gender-related factors that may influence infection rates.

Conclusion:

The present study has effectively discovered the prevailing bacterial isolates in pus cultures conducted in a rural tertiary care hospital are *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. The proportion of Grams -negative bacteria in pus cultures was shown to be greater compared to Grams positive bacteria. The bacterial isolates had the highest efficacy when treated with vancomycin, linezolid, and ciprofloxacin. The growing prevalence of *Methicillin-resistant Staphylococcus aureus* (MRSA) and *Enterococcus* is a matter of apprehension, given that these bacterial strains might present difficulties in the treatment and control of illnesses. It is essential to acknowledge that the effectiveness of Antibiotics against a particular strain of bacteria may not necessarily translate to other strains of bacteria. Hence, it is crucial to conduct Antibiotic susceptibility testing on the bacterial strains prior to commencing therapeutic intervention. The findings of this study can serve as a valuable resource in informing the choice of Antibiotics for the management of bacterial infections. Further more, these findings may be utilized to formulate focused preventative and intervention approaches for the demographic cohorts that exhibit the highest vulnerability to transmission.

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