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RESEARCH ARTICLE

Prevalence of Bacterial Pathogens and Antibiotic Susceptibility in Wound Infections: A Microbiological Study

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Abstract

Infections due to antibiotic resistant bacteria have increased alarmingly in both developed and developing countries. Unrestrained and rapidly spreading bacterial growth has turned the management of wound infection into a serious challenge. This study aimed to determine the prevalence of different bacterial pathogens and their antibiotic susceptibility in various types of samples sent to the microbiology laboratory.

A study was conducted on 110 samples collected in forms of swabs and culture. All isolated bacteria were identified based on colony characteristics, Gram staining and standard biochemical tests and antibiotic susceptibility testing with the Kirby- Bauer test, known as the disc diffusion method. This method relies on the inhibition of bacterial growth measured under standard conditions. Several statistics and pie charts are used to present observation of the study.

The rate of isolation of bacteria was 100% from the samples collected from different sites of different patients. *Staphylococcus aureus* (50.91%) was found to be the most frequent isolate, followed by *Escherichia coli* (24.55%), *Pseudomonas species* (10.91%), *klebsiella species* (5.45%), *Streptococcus pyogenes* (5.45%), *Proteus species* (2.73%).

Gram-positive where mostly found sensitive to antibiotics imipenem, gentamycin, ceftriaxone vancomycin, Azithromycin in the study. Gram-negative where mostly found sensitive to ceftazidime, Ceftriaxone, gentamicin.

The diversity of isolated bacteria and their susceptibility patterns signify a need to implement a proper infection control strategy which can be achieved by carrying out antibiotic sensitivity test of the isolates.

Key Words: *Sample; Bacteria; Isolate; Susceptibility*

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Introduction

A wound is a breach in the skin and the exposure of subcutaneous tissue after loss of the skin integrity, thus providing a moist, warm and nutritive environment that is conductive to microbial colonization and proliferation [1]. Wounds follow the loss of skin integrity, which provides a moist, warm and nutritive environment that is known to be conductive to microbial colonization and proliferation. Wound infections are considered a major complication of surgery and can be classified into three types: incisional surgical wounds, deep incisional wounds and organ-specific infections. Despite maintaining the high standards of preoperative preparation, antibiotic prophylaxis and operative procedures, the appearance of post-operative wound infections remains a grave threat among the clinicians. Some of the most frequent causative microorganisms are related to wound infections and include *Staphylococcus aureus, Streptococcus pyogenes, Enterococci, Escherichia coli, klebsiella pneumonia, Proteus* species and *Pseudomonas aeruginosa*. Surgical Site Infections (SSIs) are divided into the following categories upon assessment at 30 days after surgery: incisional SSIs-they can be superficial and involve only the skin and subcutaneous tissue of the incision, with physical findings of inflammation, deep incisional SSIs, which are also defined at 30 days post-surgery or at one year if an implant is involved and that involve infection present in the deep soft tissues of the incision; and finally, organ or space SSIs, which involve any part of the anatomy other than the incision itself [2].

However, the severity of complication is largely based on the virulence of the infecting pathogen and the site of infection. The reporting trend of infection varies depending on the surgeon's ability, operative area, surgical procedures, patient's characteristics etc. For instance, approximately 5,00,000 infections per year takes place in the United States among an estimated 27 million surgical procedures. The incidence of hospital-based postoperative infections varies from 10%-25% in India. Nosocomial infection is becoming a serious problem affecting hospitalized patients both in developed and developing countries. According to a study conducted in Bangladesh, it was reported that among 38% of nosocomial infections, more than 50% were due to wound infection. Moreover, wound infections were found to be higher (49%) among post-operative patients as compared to pre-operative patients (15.9%) in that study. Post-operative wound infections have emerged as one of the important causes of morbidity among the hospitalised patients.

Wound infection is becoming a major concern among patients and healthcare practitioners for its increased toll on morbidity and financial loss. It also generates demand for attaining expensive management within the public health system. Active and passive surveillance of surgical site infections in the hospital will help the surgeons and clinicians to know the antibiotic susceptibility pattern is related to the surgical site, which can help reduce post-operative complications. Since wound colonization is most frequently polymicrobial involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected [3].

There is often a lack of comprehensive and up-to-date data on the specific bacterial pathogens associated with wound infections in the local or regional context. The variability in antibiotic susceptibility patterns across regions and over time means existing global or national data might not adequately represent local trends. A critical gap exists in understanding the emergence of Multidrug-Resistant (MDR)

strains in wound infections, which complicates treatment strategies.

The study likely focused on local epidemiology by examining wound infections in a specific healthcare setting, identifying pathogens prevalent in that area. It provided critical insights into the antibiotic susceptibility profiles of these pathogens, shedding light on the extent and pattern of resistance to commonly used antibiotics. Highlighted any distinct patterns of resistance unique to the study area, such as the prevalence of MDR organisms. By addressing these gaps, the study contributes valuable information for guiding empirical antibiotic therapy and updating local antibiotic stewardship policies to mitigate resistance challenges.

Objectives of antibiotic susceptibility testing:

- It guides the clinician in choosing the right antibiotic for a particular infection.
- It helps in identifying the susceptibility patterns of common isolates in a particular hospital or a community.
- This data can help in choosing the right empirical treatment for critically ill patients even before their culture results are obtained from the microbiology laboratory.
- The test is performed in a microbiology laboratory under standard conditions so the results are reproducible.
- Pre-requisite for any antimicrobial susceptibility test is the presence isolated colonies of bacteria which are obtained from various specimen sent to the microbiology laboratory.

Materials and Methods

Sample collection

One hundred and ten (110) samples of wound were collected by sterile syringe and by swabs from outpatient and inpatient from department of Surgery, Gynaecology and orthopaedic wards of Bokaro General Hospital (BGH) with proper standard protocols and ethical guidelines and sent to the microbiology department of Bokaro General Hospital for antibiotic susceptibility pattern testing.

Specimen collected is typically by nurses or technician before the wound cleaning and before application of any antiseptic solution. At the time of swag collection standard care was taken to avoid any contamination by the normal flora of the surrounding skin. The specimen was loaded in transport medium and transported within one hour to the microbiology laboratory of the hospital to perform the culture and antibiotic susceptibility test.

Bacterial isolation

Subsequently specimen was in a curated on appropriate agar media: blood agar, MacConkey agar, nutrient agar and Mannitol Salt Agar. The cultures were incubated aerobically at 37 degrees Celsius for 24-48 hours with proper care. All the plates were regularly inspected for growth and identification of the isolated bacteria with done by colony morphology, Gram staining and standard biochemical tests by the microbiologists and other demonstrators in the microbiology laboratory.

Antibiotics used

The choice of antimicrobial disks to be used in the susceptibility test will depend on the

- Pathogen
- Specimen
- Range of locally available antimicrobials
- Prescribing policies of the hospital

This disc can be purchased commercially.

The supply stock of disks should be stored at -20 degree Celsius.

The disc in use should be stored in refrigerator.

Before using the disc, it should be kept outside for 20 - 30 minutes at room temperature.

1. Amoxicillin 10 mg
2. Penicillin 10 mg
3. Vancomycin 30 mg
4. Azithromycin 15 mg
5. Cephradine 30 mg
6. Tetracycline 30 mg
7. Cloxacillin 5 mg
8. Co-trimoxazole 23.75 mg
9. Ciprofloxacin 5 mg
10. Ceftriaxone 5 mg
11. Nitrofurantoin 300 mg

Different methods of antibiotic susceptibility testing

- Disc diffusion method

1. Kirby-Bauer disc diffusion method
2. Stocks disc diffusion method

- Dilution method

1. Agar dilution method
2. Broth microdilution method

- Gradient diffusion method for E-Test
- Automated antimicrobial susceptibility testing systems

There are various methods of antibiotic susceptibility testing but here we limited ourselves to the Kirby Bauer disc diffusion method in our microbiology laboratory as

- It is a simple
- Reliable
- Widely used method

Antibiotic susceptibility test: Kirby-Bauer disc diffusion Method

Principle

- Antibiotic impregnated filter paper discs are placed on a Mueller Hinton agar with lawn culture of an organism.
- The antibiotic diffuses from the disc into the anger and decreasing amounts as we move further away from the disc.
- If the organism is killed or any better by the concentration of the antibiotic there will be no growth in the immediate area around the disc. This zone is called the zone of inhibition.
- After incubation at 35 degrees Celsius for 16 to 18 hours zone size is measured and interpreted using CLSI standards which are available as charts from various manufacturers.

Various steps followed during the experiment

Part 1

- Follow proper laboratory rules.
- At the beginning of each week a nutrient broth or agriculture should be prepared for daily use in the microbiology laboratory.
- The prerequisite for any antimicrobial sensitivity test is the presence of isolated colonies of bacteria which are obtained from various specimen sent to the laboratory.

Part 2

- We culture the specimen on appropriate media.
- Incubate them overnight to get the isolated colonies of bacteria whose sensitivity of various antimicrobials can now be tested.

Part 3

- Remove the antimicrobial discs and the Mueller Hinton agar plates from the refrigerator and keep them outside for 20 to 30 minutes till they reach the room temperature.
- Now we wear personal protective

equipment's (masks, gloves) before performing the experiment.

- Label the test tubes of nutrient broth in which the colonies are to be inoculated.
- Now using a sterile wire loop, we touch 3-5 well isolated colonies of similar appearance to the test organism and emulsify in 3-4 ml of sterile nutrient broth or saline and mix it properly.
- Culture grown from a specimen can give a mixture of colonies which are representative of different bacteria present in the specimen. Cash should be taken to pick only one type of colony.
- Then we match the turbidity of the suspension to the 0.5 Mac Farland turbidity standard in good light. [Mac Farland is a barium sulphate standard against which the turbidity of the test and control inocula can be compared. This process will give the inocula confluent growth. This standard should always be shaken well before use].
- Then we take Mueller Hinton agar plates and sterilize it as directed by the laboratory incharge.
- The pH of the medium should be check, that is, 7.2-7.4.
- The depth of the agar medium can affect the test accuracy. If the medium is too thick the zones will be falsely small and if too thin then the inhibition zones will be falsely large.
- We generally for about 25 ml of media into 90mm diameters petri plates to give a depth of 4mm.
- Care must be taken to put the plates on a level surface so that the death of the medium is uniform.

Part 4

- Label the base of the Mueller Hinton agar plate with sample details.
- Using a sterile swab inoculate a labelled plate of Mueller-Hinton plate by the lawn culture method.
- With the petri dish in place allow 3-5 minutes (no longer than 15 minutes) for the surface of the agar to dry.
- Using sterile forceps, take the antimicrobial disc and place the disc appropriately on the inoculated agar plate.

(Note: The disks should be about 15 mm away from the edge of the plates and no closer about 25mm to disk to disk)

- No more than 6 discs to be applied on a 90 mm petri plate.
- We also inoculate control plates of Mueller Hinton agar with control strains of *E. coli* ATCC25922 for gram-negative bacteria.
- *Staphylococcus aureus* ATCC25923 for gram-positive bacteria.
- We label the petri plates properly and let them incubate

Note: The Mueller Hinton agar plate should be from the same batch as the test plates and the antimicrobial this should be similar to the ones applied on the test plates. Within 30 minutes on applying the disks invert the control and the test plates and incubate them aerobically at 35 degrees Celsius for 16-18 hours.

Part 5

- After overnight incubation examine the control and the test plates to ensure the growth is uniform.
- Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm.

Interpretation of zone sizes

The interpretive categories have been standardized and define in CLSI guidelines "Performance standards of AST" (Table 1).

TABLE 1
Interpretation of zone sizes.

Susceptible (S)	Intermediate (I)	Resistant (R)
Interpretive category that indicates an organism is inhibited by the recommended dose of an antimicrobial agent at the infection site.	Interpretive category that represents an organism that may require higher dose of antibiotic for a longer period of time to be inhibited.	Interpretive category that indicates an organism is not inhibited by the recommended dose of an antimicrobial agent at the infection site.

On basis of this chat, we report the organism as 1) Resistant(R) 2) Intermediate/ Moderately susceptible(I) 3) Sensitive (S)

We repeated this with many samples.

Results

Out of total 110 study sample 56.36% of culture positive plates turned out to be gram-positive organisms and 43.64% gram-negative organisms.

Staphylococcus aureus (N= 56; 50.91%) was predominantly found to be isolated among all the representing bacteria, followed by *Escherichia coli* (N=27; 24.55%) (Tables 2 and 3).

Staphylococcus aureus > *Escherichia coli* > *Pseudomonas* species > *Streptococcus pyogenes* > *klebsiella* species > *Proteus* species (Figures 1 and 2).

TABLE 2
Number and percentage of gram-positive bacteria isolated.

Gram-positive bacteria (N= 62; 56.36%)	Number	Percentage
Staphylococcus aureus	N=56	50.91%
Streptococcus pyogenes	N=6	5.45%

TABLE 3
Number and percentage of gram-negative bacteria isolated.

Gram-negative bacteria (N=48; 43.64%)	Number	Percentage
Escherichia coli	N= 27	24.55%
Klebsiella species	N= 6	5.45%
Pseudomonas species	N=12	10.91%
Proteus species	N=3	2.73%

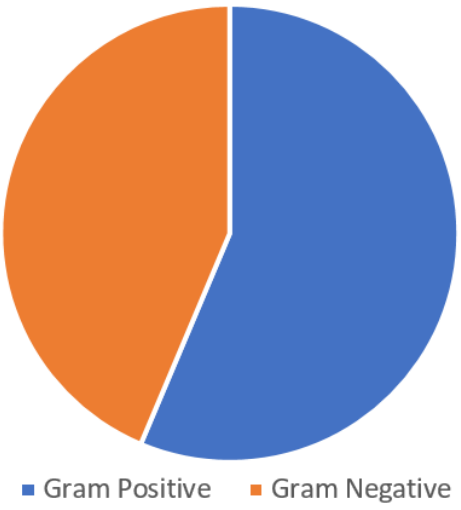

Figure 1) *Pattern of bacterial growth among total samples (N=110).*

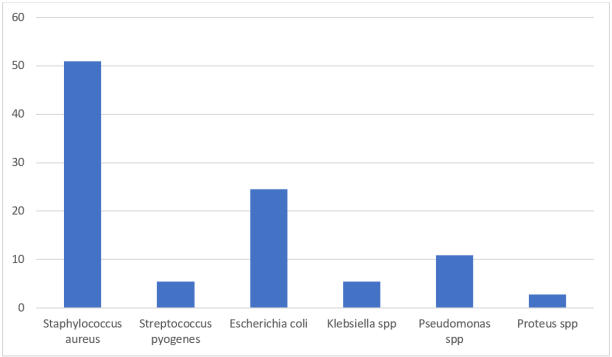

Figure 2) *Rate of isolation of different bacteria (N=110).*

Sensitivity patterns of isolated gram-positive and gram-negative bacteria

The susceptibility pattern of gram-positive bacteria was mostly sensitive to imipenem (90%), followed by ceftriaxone (85.5%), gentamicin (81.8%), vancomycin (80.8%), Azithromycin (76.5 %) and other antibiotics (<75%).

Most of the gram-negative isolates were sensitive to ceftazidime (79%) ceftriaxone (71.8 %), gentamicin (7.7 %) and other antibiotics (<70%) (Tables 4 and 5).

TABLE 4
Sensitivity patterns of gram-positive bacteria (N=62).

Antimicrobial agents	*Staphylococcus aureus* (N = 56)	*Streptococcus pyogenes* (N=6)
Amoxicillin 10 mg	33	3
Penicillin 10 mg	32	3
Vancomycin 30 mg	41	5
Azithromycin 15 mg	42	4
Cephradine 30 mg	32	2
Tetracycline 30 mg	30	3
Cloxacillin 5 mg	31	3
Cotrimoxazole 23.7 mg	31	2
Gentamicin 10 mg	42	4
Ciprofloxacin 5 mg	32	3
Cefixime 5 mg	38	3
Cefuroxime 30 mg	33	4
Imipenem 10 mg	53	5
Ceftriaxone 30 mg	47	5

TABLE 5
Sensitivity patterns of gram-negative bacteria (N= 48).

Antimicrobial agents	*Escherichia coli* (N=27)	*Klebsiella species* (N=6)	*Pseudomonas* species (N=12)	*Proteus* species (N=3)
Cephradine 30 mg	11	0	0	0
Cotrimoxazole 23.7 mg	14	2	3	2
Cefixime 5 mg	21	2	3	1
Penicillin 10 mg	9	0	2	0
Aztreonam 30 mg	18	1	1	2
Cloxacillin 5 mg	13	0	0	0
Cefuroxime 30mg	19	0	0	1
Tetracycline 30mg	16	0	4	1
Imipenem 10 mg	21	2	3	2
Ceftriaxone 30 mg	25	2	6	3
Ciprofloxacin 5 mg	7	0	4	0
Azithromycin 15 mg	10	2	3	1
Amoxicillin 10 mg	2	1	0	0
Cefotaxime 30 mg	22	1	0	1
Gentamicin 10 mg	24	6	6	1
Ceftazidime 30 mg	23	6	5	2
Nitrofurantoin 300 mg	17	1	1	1

Discussion

As per literature about wound infections, surgical site infection rate is about 3.03% in clean surgeries and 22.41% in clean-contaminated surgeries. Significant increase can be found in surgical site infection rate with an increase in preoperative stay. The increase in duration of surgery is associated with a significant rise in the rate of surgical site infection. Surgical site infection rate is much higher (22.41%) in cases where a drain is used than in non-drained wounds (3.03%) [4]. Among all nosocomial infections, Nosocomial urinary tract infections make up usually 42% of the infections, surgical wound infections about 24%, nosocomial pneumonia 10%, nosocomial bacteraemia 5% and nosocomial infections at all other sites make up about 19% [5].

From the present study it may be concluded that the predominant isolation from bone infection was *Staphylococcus aureus* followed by *Escherichia coli*, *Pseudomonas* species and *Proteus* species. Alarmingly high rate of resistance to commonly used antibiotics was observed. The isolates were highly resistant to Amoxicillin, ciprofloxacin, tetracycline and clotrimoxazole. While they were fairly sensitive to imipenem, gentamicin and ceftriaxone. Results match this of other studies where independent of sites, common bacteria isolated were *E. coli* and *S. aureus* [6]. Continuous monitoring and surveillance will help the creation and appropriate antibiotic selection and proper management of wound infection. Judicial and rational use of antibiotics should be sought to prevent the emergence of resistant pathogens.

The susceptibility data from this report may be worth consideration while implementing empiric treatment strategies for pyogenic infections. At the same time, strict health policies should also be implemented to regulate the purchase and prescription and district the unsupervised antibiotic use as well as continuous monitoring and reporting antibiotic resistance.

Conclusion

This study highlights the prevalence of bacterial pathogens in wound infections, with gram-positive organisms (56.36%) being more common than gram-negative ones (43.64%). *Staphylococcus aureus* emerged as the predominant isolate (50.91%), followed by *Escherichia coli* (24.55%).

Understanding the distribution of pathogens and their antibiotic susceptibility is crucial for guiding effective treatment strategies, reducing infection-related complications and combating the growing threat of antimicrobial resistance. Such research is essential for developing targeted interventions and improving patient outcomes in clinical settings.

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