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Bacteriological profile of pyogenic infections at a Tertiary Care Centre of Nepal

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ABSTRACT

Background: The pyogenic infections includes a wide range of abnormalities like superficial skin infections, eyes infections, wound infections, infection of burns, boils, furuncles, peritonitis and abscesses. Some of the infections are endogenous that occurs by the patient's own normal flora. Many infections are exogenous that occur by direct and indirect airborne routes. Boils and furuncles are caused by *Staphylococcus aureus.* Gram negative infections rarely occur on healthy skin except moist area of skin and axilla. **Aims and objectives:** The purpose of this study was to illustrate the bacteria responsible for pyogenic infection and to determine their antibiotic susceptibility. **Methods:** The pyogenic bacteria were isolated from the samples collected from the Clinical Departments of Chitwan Medical College. The isolates were identified and antibiotic susceptibility test was performed by standard protocols. **Results:** Gram negative bacteria were frequently isolated pathogens than the Gram positive bacteria. *Escherichia coli* (*E..coli*) was the predominant isolate among the 138 positive samples, 49 (35.5%) of them was only the *E.coli*, followed by *Staphylococcus aureus* (15.21%), *Klebsiella pneumoniae (K. pneumoniae)* (13.04%), *Acinetobacter* species (11.59%), MRSA (11.59%), *Pseudomonas aeruginosa* (5.79%), *Klebsiella oxytoca* (3.62%), Enterobacter (2.17%), and *Proteus mirabilis* (1.44%). Gram negative bacteria were highly susceptible to Amikacin whereas most of the Gram-positive isolates were susceptible to vancomycin and linezolid. **Conclusion:** The knowledge of the most prevalent type of bacterial isolates and their antimicrobial susceptibility pattern is a must for the clinicians as it aids in the accurate selection of the therapeutic regimens.

Introduction

Pyogenic bacteria whenever aggress with the human immune system, a viscid pus is produced as there is the release of leukocidins that kills the neutrophils [1]. This represents the typical infection of *Staphylococcus aureus (S.aureus).* Pyogenic bacteria involve in the formation of pus or postules at the site of abscesses or any types of inflammation. The pigmentation of bacteria determines the color of the pus. Pyogenic bacteria may be either Gram negative or Gram positive, aerobes or facultative aerobes [2]. The Surgical or accidental wounds have a tendency to be infected by *S.aureus*, common multiple drug resistant bacteria in hospital settings. Streptococcal infection includes cellulitis, impetigo, erysipelas, ecthema and scarlet fever too. Moreover, Gram negative bacteria cause ocular infections, intestinal disesases, cardiac diseases and throat infections. Further blood stream infections,

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meningitis, surgical site infections might be the cause of Gram negative bacteria.

Gram negative bacteria are resistant to the multiple antibiotics available in this era. They have in-built capacity of becoming resistant to most of the drugs. This inbuilt capacity passes along with the genetic materials and makes other bacterial pathogen to be resistant to the available drugs. This has created a serious threat to the human health. Detection of the pyogenic organisms could be the mainstay of early and accurate diagnosis of the infected sites and it also aids in the accurate prescription of therapeutic regimens or the treatment planning of pyogenic infections [3].

The pyogenic bacteria can be cultured on various culture media or agar plates. The colony characteristics that differ from one bacterium to other can aid in the identification of pyogenic bacteria. Moreover, several rapid diagnostic tests, serological tests like ELISA and molecular methods like polymerase chain reaction; electrophoresis helps in the identification of pyogenic organisms.

The mainstay of treatment of pyogenic infection includes the surgery and the use of antibiotics prescribed on the basis of system or organ affected [4]. These antibiotics have a bacteriostatic or bactericidal effect on the bacteria. Thus, this study signifies the changing trend of antibiotic resistance among the bacterial isolates.

Objectives

This study is aimed to isolate and identify the bacterial strains isolated from pyogenic infections and to determine their drug susceptibility pattern of the common antibiotics used in therapeutic management.

Materials and Methods

Sample collection

A total of 200 pus samples were aseptically collected by sterile syringe aspiration (n = 48) and by sterile swabs (n =152) from inpatients and outpatients of various wards of Chitwan medical college and Teaching Hospital (CMCTH), Bharatpur, Nepal. The study was conducted during a period of 3 months from October 2020 to December 2020 with the standard protocols and was permitted by the Ethical committee of CMCTH. The pus samples collected from various clinical departments were aseptically transferred to the Cary Blair transport media and transported to the lab avoiding any types of microbial contamination.

Characterization and identification of pyogenic bacteria

The samples collected were processed for bacterial culture on CLED agar (Cysteine Lactose Electrolyte Deficient Agar), 5% sheep blood agar and MacConkey agar to isolate the organism present in the sample. Further the samples were also subjected for Gram staining to determine the presence of either Gram negative or Gram positive bacteria. The culture plates were incubated at 37 degree Celsius aerobically for 24-48 hours. After the incubation period, the colony characteristics (colour, shape, transparency, consistency, etc.) were noted for any growth seen. The grown bacterial isolates were subjected for several biochemical tests such as SIM (Sulphide Indole Motility), TSI (Triple Sugar Iron Agar), citrate utilization test , urea hydrolysis test, catalase test, and oxidase tests to identify the bacterial isolates.

Antibiotic susceptibility test

An inoculum of bacterial isolates was made on Mueller Hinton agar (MHA) before which the turbidity was adjusted to 0.5 McFarlands standards. The inoculum was spread to the whole MHA plate by a clean, dry and sterile glass spreader. The antibiotics were taken out from the disc container by a sterile forcep and placed to the MHA plate. The fork was slightly pressed onto the media such that the antibiotic disc could properly touch to the media. The plates inoculated with bacterial isolates and antibiotic disc were incubated at 37 degree Celsius for 18-24 hours. After then, the plates were observed to determine the susceptibility pattern. The diameter of a zone of inhibition was measured by scale for each antibiotic disc. Intermediate, sensitive and resistance pattern was determined [5].

MRSA detection

For the detection of methicillin resistance in *S.aureus* cefoxitin (30 μg) discs and oxacillin (1 μg) discs were used to confirm the presence of methicillin resistant *S. aureus (*MRSA). The zones of inhibition (ZOI) of both discs were measured. The isolates showing the ZOI of ≤21 mm with the cefoxitin disk or ≤10 mm with the oxacillin disk were identified to be MRSA as recommended by the Clinical and Laboratory Standards Institute (CLSI) [6].

Results

Among the 200 pus samples collected from various wards of the hospital, 138 samples (69%) were found to be positive after an incubation period of 24-48 hours. On the basis of colony

characteristics, microscopic characteristics, Gram staining results and biochemical tests, the pyogenic isolates were identified to be nine species.

Gram negative bacteria were the most frequent isolates comprising of 73.15% (101/138) as compared to the Gram positive isolates (26.85%). *Escherichia coli* (35.5%) was the predominant isolate followed by *S. aureus*, *Klebsiella pneumoniae* (*K.pneumoniae)* (13.04%), *Acinetobacter* species (11.59%), MRSA (11.59%), *Pseudomonas aeruginosa* (5.79%), *Klebsiella oxytoca* (*K. oxytoca*) (3.62%), *Enterobacter* (2.17%), and *Proteus mirabilis* (1.44%) as shown in **table (1)**.

The antibiotic susceptibility pattern of the bacterial isolates from **table (2)** shows that Gram negative bacteria were highly sensitive to amikacin (*E.coli, Enterobacter species, K. pneumoniae, K. oxytoca, Acinetobacter species, Proteus mirabilis and Pseudomonas aeruginosa*). Among all the Gram negative bacterial isolates, *K. oxytoca* was highly sensitive to cotrimoxazole and levofloxacin. *Proteus mirabilis* was highly sensitive to piperacillin-tazobactam, cefotaxime and ciprofloxacin. *Klebsiella oxytoca* was highly sensitive to levofloxacin, ceftriaxone and tigecycline. All the Gram negative isolates were highly resistant to Amoxycillin-clavulanic acid. Enterobacter species was highly resistant to piperacillin-tazobactam and ceftriaxone.

The antibiotic susceptibility pattern of the Gram positive isolates depicts that *S. aureus* was highly sensitive to amikacin, vancomycin and teicoplanin whereas extensively resistant to erythromycin. MRSA was extensively sensitive to linezolid, vancomycin and teicoplanin whereas extremely resistant to cefotaxime and ciprofloxacin. Both Gram-positive isolates were fully susceptible to vancomycin and linezolid as shown in **table (3)**.

Table 1. Frequency of isolates in pus samples.

Isolates	**Number**	**Percentage**
Escherichia coli	49	35.5
Klebsiella pneumonia	18	13.04
Klebsiella oxytoca	5	3.62
Acinetobacter species	16	11.59
Proteus mirabilis	2	1.44
Pseudomonas aeruginosa	8	5.79
Enterobacter species	3	2.17
Methicillin Resistant *Staphylococcus aureus* (MRSA)	16	11.59
Staphylococcus aureus	21	15.21
Total	138	100

Table 2. Antibiotic susceptibility pattern of the Gram-negative pyogenic isolates.

Antibiotic	*Escherichia coli* (N=49)		*Enterobacter species* (N=3)		*Klebsiella pneumonia* (N=18)		*Klebsiella oxytoca* (N=5)		*Acinetobacter species* (N=16)		*Proteus mirabilis* (N=2)		*Pseudomonas aeruginosa* (N=8)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Cotrimoxazole	18 (36.7)	31 (63.3)	1 (33.3)	2 (66.7)	11(61.1)	7 (38.9)	4 (80.0)	1 (20.0)	4 (25.0)	12 (75.0)	1(50.0)	1(50.0)	Nt	Nt
Piperacillin/ Tazobactam	22 (44.9)	27 (55.1)	0 (0)	3 (100)	11(61.1)	7 (38.9)	3 (60.0)	2 (40.0)	2 (12.5)	14 (87.5)	2 (100)	0	2 (25.0)	6 (75.0)
Cefotaxime	14 (28.6)	35 (71.4)	1 (33.3)	2 (66.7)	11(61.1)	7 (38.9)	4 (80.0)	1(20.0)	2 (12.5)	14 (87.5)	2(100)	0	4 (50.0)	4 (50.0)
Amikacin	46 (93.9)	3 (6.1)	2 (66.7)	1 (33.3)	13(72.2)	5 (27.8)	4 (80.0)	1(20.0)	9 (56.2)	7 (43.7)	2 (100)	0	6 (75.0)	2 (25.0)
Levofloxacin	22 (44.9)	27 (55.1)	Nt	Nt	10(55.5)	8 (44.4)	5 (100)	0 (0)	6 (37.5)	10 (62.5)	0 (0)	2 (100)	6 (75.0)	2 (25.0)
Ceftriaxone	19 (38.8)	30 (61.2)	0	3 (100)	9 (50.0)	9 (50.0)	5 (100)	0 (0)	5 (31.2)	11 (68.7)	1(50.0)	1(50.0)	5 (62.5)	3 (37.5)
Tigecycline	44 (89.8)	5 (10.2)	Nt	Nt	12(66.7)	6 (33.3)	5 (100)	0 (0)	16 (100)	0 (0)	Nt	Nt	7 (87.5)	1 (12.5)
Amoxycillin/ Clavulanic acid	8 (16.3)	41 (83.7)	1 (16.7)	2 (83.3)	3 (16.7)	15(83.3)	2 (40.0)	3 (60)	0 (0)	16 (100)	1(50.0)	1(50.0)	1 (12.5)	7 (87.5)
Cefixime	10 (20.4)	39 (79.6)	Nt	Nt	10(55.5)	8 (44.4)	2 (40.0)	3 (60)	Nt	Nt	Nt	Nt	Nt	Nt
Meropenem	37 (75.5)	12 (24.5)	3 (61.1)	0 (38.9)	11(61.1)	8 (38.9)	Nt	Nt	6 (37.5)	10 (62.5)	Nt	Nt	6 (75.0)	2 (25.0)
Ciprofloxacin	Nt	Nt	Nt	Nt	18 (100)	0 (0)	Nt	Nt	Nt	Nt	2 (100)	0	Nt	Nt
Gentamycin	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	1(50.0)	1(50.0)	Nt	Nt

nt: not tested

Table 3. Antibiotic susceptibility pattern of the Gram positive pyogenic isolates.

Antibiotics	***S. aureus* (N=21)**		**MRSA (N=16)**	
	S (%)	R (%)	S (%)	R (%)
Cotrimoxazole	14 (66.7)	7 (33.3)	8 (50.0)	8 (50.0)
Piperacillin/Tazobactam	12 (57.1)	9 (42.8)	2 (12.5)	14 (87.5)
Cefotaxime	14 (66.7)	7 (33.3)	0	16 (100)
Amikacin	21 (100)	0	14 (87.5)	2 (12.5)
Levofloxacin	19 (90.5)	2 (9.5)	4 (25)	12 (75.0)
Ceftriaxone	15 (71.4)	6 (28.6)	0	16 (100)
Tigecycline	Nt	Nt	15 (93.7)	1 (6.2)
Amoxycillin/clavulanic acid	7 (33.3)	14 (66.7)	2 (12.5)	14 (87.5)
Cefixime	Nt	Nt	Nt	Nt
Meropenem	Nt	Nt	Nt	Nt
Ciprofloxacin	Nt	Nt	0	16 (100)
Gentamycin	Nt	Nt	Nt	Nt
Linezolid	20 (95.2)	1 (4.8)	16 (100)	0
Vancomycin	21 (100)	0	16 (100)	0
Teicoplanin	21 (100)	0	16 (100)	0
Ofloxacin	17 (80.9)	4 (19.0)	Nt	Nt
Cefoxitin	16 (76.2)	5 (23.8)	1 (6.2)	15 (93.7)
Cloxacillin	15 (71.4)	6 (28.6)	1 (6.2)	15 (93.7)
Clindamycin	11 (50.0)	11 (50.0)	6 (37.5)	10 (62.5)
Erythromycin	6 (28.6)	15 (71.4)	5 (31.2)	11 (68.7)
Imipenem	13 (61.9)	8 (38.1)	Nt	Nt

Discussion

This study was aimed to detect the pyogenic bacteria from the clinical pus samples and determine their drug susceptibility pattern. Gram negative bacteria were the predominant isolates in this study. Moreover, *E.coli* was the highly prevalent organism followed by *S. aureus*. Study conducted at a tertiary care hospital Puducherry by **Rameshkannan et al.** also stated *E. coli* to be the most common organism isolated from pus samples [7]. The study is also similar to the study carried out at Jinling Hospital of China by **Zhang et al**. who reports *E. coli* to be dominant bacteria followed by *S. aureus* and *K. pneumoniae* [8]. The study by **Trojan et al.** conducted at Punjab, India also reveals that *E.coli* is the predominant isolate followed by *S. aureus*, and *K. pneumoniae* which is in correlation to our study [9].

The study by **Bessa et al**. conducted at Chieti, Itlay states that *S.aureus* is the predominant isolate in wound infections followed by *Pseudomonas aeruginosa* and *Proteus mirabilis* [10]. The study carried out by **Dryden** states that *S.aureus* and MRSA, the predominant causative agents of skin infections [11].

The antibiotic susceptibility profile of the bacteria isolated from the pus samples of various in this study shows that Gram negative bacteria (*E.coli, Enterobacter species, Klebsiella pneumonia, Klebsiella oxytoca, Acinetobacter species, Proteus mirabilis and Pseudomonas aeruginosa*) were highly sensitive to amikacin which correlates with the study of **Imade et al.** [12].

Klebsiella oxytoca was highly sensitive to cotrimoxazole and levofloxacin. *Proteus mirabilis* was highly sensitive to piperacillin- tazobactam, cefotaxime and ciprofloxacin. *Klebsiella oxytoca* was highly sensitive to levofloxacin, ceftriaxone and tigecycline. All the Gram negative isolates were highly resistant to amoxycillin-clavulanic acid. *Enterobacter species* was highly resistant to piperacillin-tazobactam and ceftriaxone. The study

shows that *Pseudomonas aeruginosa* is highly resistant to amoxycillin/clavulanic acid which correlates with the various studies.

This study revealed that the potency of ciprofloxacin was high against *K.pneumoniae* and *Proteus mirabilis* whereas gentamycin has been found to be less potent against *Proteus mirabilis*. The study carried out by at Jimma University Specialized hospital, in Southwest Ethiopia found ciprofloxacin to be the most effective drug against Gram negative pathogens [13].

This study also shows that *S.aureus* and MRSA was found to be extremely sensitive to vancomycin & linezolid which agrees with studies of **Chauhan et al.** Carried out at a tertiary care centre of India [14].

According to this study Enterobacteriaceae members show high sensitivity towards amikacin and tigecycline which is not similar to the study done by **Duggal et al.** [15]. The study demonstrate that Acinetobacter strains showed high sensitivity towards tigecycline unlike the study carried out by **RaoRaghav et al.** [16] which demonstrate the *Acinetobacter species* more sensitive to piperacillin- tazobactam.

Bacteria becomes resistant clinically, naturally or in acquired mode due to the inadequate consumption of drugs, overuse, sporadic use, irregular consumption of drugs, improper diagnosis of the patients and incorrect prescription of the drugs. Eradication of these negligible errors might lead to the prevention of antibiotic resistance. Antibiotic resistance has not only created a serious threat to the physical health, but also to the mental and social health of people. It has also led to the diminishing economy of the patients. Moreover, it could also increase the mortality rate of patients with high risk diseases. Knowledge of the pyogenic microorganisms and their susceptibility pattern to different antibiotics can help the clinicians in the chemotherapy of the patients [17-20].

Conclusion

Pyogenic infections are more frequent in the developing countries. A high prevalence of antibiotic resistant isolates recovered from pyogenic infections in our settings indicates the need for the continuous supervision of drug susceptibility pattern. Antibiotic policies should be implemented to control this increasing trend of resistance among pyogenic isolates. The site of inflammation should be identified in order to get the proper and adequate therapy. The treatment of pyogenic infections is still a challenge to the clinicians, the standard microbiological procedure, antibiotic drug and surgery. There has been a increasing frequency of drug resistance in bacteria. Thus, this study was aimed to identify the pyogenic bacteria and determine their antibiotic susceptibility pattern.

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