ABSTRACT

Objective: The objective of the study is to find out the resistance pattern of pathogenic organisms isolated from intra-abdominal infection (IAI).

Methods: A total of 500 samples were collected from suspected IAI patients reporting to the hospital and cultured. Identification of the isolates was done using standard identification protocol. Antimicrobial susceptibility was performed by Kirby-Bauer disc diffusion method and interpretation was done using Central Laboratory Standard Institute guidelines.

Results: Out of 500 samples, 170 were culture positive and 330 showed no growth. Gram-negative organisms (n=127) outnumbered the Gram-positive organisms (n=23). Among the Gram-negative organisms, Escherichia coli (n=67) was the most commonly isolated bacilli followed by Klebsiella sp. (n=32), Pseudomonas sp. (n=25), Acinetobacter baumannii (n=18), and Klebsiella oxytoca (n=10). Among Gram-positive organisms Staphylococcus aureus (n=17) and Enterococcus spp (n=06) isolates of were grown in culture. Among Gram-negative bacilli, Imipenem followed by Gentamicin was the most effective drug but in Acinetobacter spp. The second most effective drug was Tigecycline. Among gram-positive isolates, Linezolid was the most effective drug.

Conclusion: Prompt starting of empirical antimicrobials based on the local susceptibility pattern, followed by modification of treatment in accordance with the antimicrobial susceptibility report can significantly reduce the morbidity and the mortality associated with IAI.

Keywords: Intra-abdominal infections, Methicillin-resistant Staphylococcus aureus, Empirical antimicrobials, Emerging resistance, Gram-negative organisms, Gram-positive organisms.

INTRODUCTION

Intra-abdominal infections (IAIs) are associated with significant morbidity and mortality and common cause of hospitalized patients [1]. IAIs is a wide term that encompasses a number of infectious processes which include peritonitis, diverticulitis, cholecystitis, cholangitis, pancreatitis, chronic liver failure, and intestinal perforation [2]. According to the Infectious Diseases Society of America, complicated IAIs are associated with higher mortality rates because of compromised patient’s immunity due to underlying illness and infections with multidrug-resistant organisms [1]. Antimicrobial management plays a vital role in critically ill patients with IAI as selection of wrong antimicrobial can cause therapeutic failure which can lead to further mortality rate [6]. Initially, empirical therapy should be started based on the most frequently isolated organism and local pattern of antibiotic susceptibility, which should be modified to specific antimicrobials after receiving the microbiology report of antimicrobial susceptibility of the isolated organism [7]. Growing emergence of resistance to multiple drugs and dearth of local data on antimicrobial resistance pattern of IAI is the cause of concern for the management of IAI’s [8]. By keeping in mind the above facts, the present study was planned to determine the resistance pattern of pathogenic organisms isolated from IAI's.

METHODS

The present study was conducted for the duration of 2 years, i.e., 2018–2020 to determine the bacteriological profile and antimicrobial susceptibility pattern of isolates from the samples received from IAIs in the department of Microbiology, MMIMSR, Mullana, Ambala, Haryana, India. Ethical clearance for the study was taken from Institutional Ethical Committee.

Processing of samples

A total of 500 samples, like - ascitic fluid, bile, pus from intra-abdominal cavity, were obtained from patients suffering from IAs such as peritonitis, cholelithiasis, appendicitis, pancreatitis, liver abscess. All the samples were cultured on Blood agar and MacConkey agar and incubated at 37°C for 18–24 h and gram staining was performed on each sample. Identification of isolates was done using standard identification protocol (Mackey and McCartney) and other relevant biochemical tests as appropriate for isolates. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion methods and as per Central Laboratory Standard Institute (CLS), 2018 guidelines [9-11].

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Antibiotic susceptibility tests

Antimicrobial sensitivity pattern of the isolates was performed using Kirby-Bauer disk diffusion method and interpreted according to guidelines of CLSI. Antimicrobial sensitivity testing for Gram-negative isolates was applied using:
a. For *Escherichia coli*, *Klebsiella spp.* and *Pseudomonas oxxytoca*: Ciprofloxacin (5 µg), Piperacillin-Tazobactum (100/10 µg), Amikacin (30 µg), Ceftrixone (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Cefepime (30 µg), Trimethoprim-Sulphomethoxazole (1.25/23.75 µg).
b. For *Pseudomonas spp.* and *Acinetobacter baumanii*: Ciprofloxacin (5 µg), Levofloxacin (5 µg), Gentamicin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Cefepime (30 µg), Ceftaxidime (30 µg), Netilmicin (30 µg), Tobramycin (10 µg), and Ticarcillin-Clavulanic acid (75/10 µg).

In addition, Amikacin (30 µg) was also tested against *Pseudomonas spp.*
For *A. baumanii*: Ceftriaxone (30 µg), Trimethoprim-Sulphomethoxazole (1.25/23.75 µg), Piperacillin-Tazobactum (100/10 µg), Minocycline (30 µg), and Tigecycline (15 µg) were also additionally tested.

Antibiotics applied for Gram-positive isolates were: Ampicillin (10 µg), Erythromycin (15 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Tetracycline (30 µg), and Linezolid (30 µg).

Additional antibiotics tested against *Staphylococcus aureus* were Penicillin (10 units), Ceftazidime (30 µg), Gindamycin (2 µg), Trimethoprim-Sulphamethoxazole (1.25/23.75 µg), Minocycline (30 µg), and Doxycycline (30 µg).

Additional antibiotics tested against *Enterococcus spp.* was High-level Gentamicin (120 µg).

Screening for Methicillin-resistance in *S. aureus* was done using cefoxitin disc as per CLSI guidelines.

RESULTS

Out of 500 samples collected from clinically suspected cases of IAIIs, 405 were indoor patients while 95 were outdoor patients (Table 1). All 500 samples were cultured out of which, 330 showed no growth while growth was obtained in 170 samples. Out of 170 samples, 23 samples showed growth of Gram-positive cocci, in which *S. aureus* (n=17) were predominant followed by *Enterococcus spp.* (n=6). Among Gram-negative isolates fermentative bacilli (n=104) were predominant as compared to non-fermentative bacilli (n=43). Out of all fermentative bacilli *E. coli* was predominant (n=67) followed by *Klebsiella spp.* (n=32) and *K. oxytoca* (n=5). In non-fermentative bacilli, *Pseudomonas spp.* (n=25) were more followed by *Acinetobacter baumanii* (n=18) (Table 2).

The most predominant IAIIs were Peritonitis and Intestinal perforation in which most isolated organisms were *E. coli* followed by *Klebsiella spp.* and *Pseudomonas spp.* (Table 3).

Table 1: Distribution of patients from IPD and OPD included

| Total no. of samples | IPD  (81%) | OPD (19%) |
|----------------------|------------|-----------|
| 500                  | 405        | 95        |

IPD: Inpatient department, OPD: Outpatient department

**Table 2: Total samples showing growth and no growth in clinically suspected cases of IAIIs**

| Total no. of samples cultured | No growth | With growth (n=170) |
|-------------------------------|-----------|---------------------|
|                               | GPC* | GNB* fermenters | GNB non-fermenters |
| 500                           | 330  | 23  | 104 | 43 |

*Gram-positive Cocci,* *Gram-negative Bacilli

The Anti-microbial susceptibility testing was also performed in which Imipenem was found to be the most effective drug among all Gram-negative isolates followed by Gentamicin except for *Acinetobacter baumanii* in which the second most effective drug was Tigecycline (Table 4).

All the *S. aureus* (n=17) isolated were resistant to Cefoxitin, Penicillin and Oxacillin while highly sensitive to Vancomycin, Linezolid (100%) followed by Teicoplanin (85%). Among *Enterococcus spp.*, Vancomycin and Linezolid (100%) were the most sensitive antibiotic followed by Teicoplanin (91%) (Table 5).

**DISCUSSION**

The emergence of resistance to routinely used antibiotics and even to newer antibiotics has made the treatment of IAIIs a real challenge as a result accurate laboratory cultures for identification of organisms and their sensitivity testing has to be done with standard protocols. So that the microbiologists and physicians can go to decrease the mortality rate due to IAIIs.

In the present study, Inpatient department (IPD) 405 (81%) patients were predominant over Outpatient department (OPD) 95 (19%) patients which are suspected IAIIs (Table 1). This correlates with the study done by Jangla et al. which showed (76%) of the patients from IPDs and (24%) were from OPDs [12].

The rate of positivity from our study was 170/500 (34%) in which Gram-negative isolates (n=147) were dominating over Gram-positive isolates (n=23) (Table 2). Among the Gram-negative bacilli, the major isolates were of *E. coli* (n=67) followed by *Klebsiella spp.* (n=32), *K. oxytoca* (n=5) in fermenters and *Pseudomonas spp.* (n=25) followed by *A. baumannii* (n=18) in Non-fermenters. In Gram-positive isolates *S. aureus* (n=17) were more than *Enterococcus spp.* (n=6). These findings correlate with study done by Zhang et al. in which *E. coli* (47.3%) is the most common isolate obtained followed by *Klebsiella spp.* (17.2%). *Pseudomonas spp.* (10.1%), *A. baumannii* (8.3%), *K. oxytoca* (1.8%) [13]. Garg et al. conducted the same study and revealed that the *E. coli* (27.6%) is the most common isolate obtained in culture-positive samples of IAIIs followed by *Klebsiella spp.* (16.9%), *A. baumannii* (12.5%), *Pseudomonas spp.* (8.9%) [5] (Table 3).

In the present study, Peritonitis (n=68) was found to be predominant clinical conditions followed by intestinal perforation (n=46) including Cholelithiasis (n=20), Appendicitis (n=15), Pancreatitis (n=10), and Liver abscesses (n=9), respectively (Table 3). According to study done by Sukanya et al. peritonitis perforation (36.1%) was predominant clinical conditions followed by acute pancreatitis (11.7%) and necrotic pancreatitis (10%) [14].

The Anti-microbial susceptibility testing was also performed in which Imipenem was found to be the most effective (*E. coli* 92.5%, *Klebsiella spp.* 93%, *K. oxytoca* 91%, *Pseudomonas spp.* 94% and *A. baumannii* 88%) drug among all Gram negative isolates followed by Gentamicin (+90%) except for *A. baumannii* in which second most effective drug was Tigecycline (82%) (Table 4). Similar results were found in study done by Shree et al. showed that the antibiotic resistance rate of Gram-negative isolates to Carbapenem, (Ertapenem – 29–41%) and (Meropenem – 14–15%) Imipenem, Tigecycline, and Colistin were most sensitive [15].

In the present study, all the *S. aureus* (n=17) isolated were resistant to Cefoxitin, Penicillin while highly sensitive to Linezolid (100%) (Table 5). Overall *Enterococcus spp.* was also found highly susceptible to Vancomycin and Linezolid (100%). In accordance of this Shree et al. also stated that *S. aureus*, higher resistance is shown to penicillin (91.7%), *ciprofloxacin* (58.4%), and gentamicin (33.3%) however, *S. aureus* is 100% sensitive to Vancomycin and Linezolid [15]. Similarly according to study done by Garg et al. revealed 100% sensitivity to Vancomycin and Linezolid, 91.66% to Amikacin and Cidamycin each, Cotrimoxazole 75%, Erythromycin 56.33%, and *Ciprofloxacin* 50% [5].
Knowing the prevalent pattern of antimicrobial resistance is an important issue especially when Gram-negative isolates continue to exhibit widespread resistance to various currently in use antimicrobial agents. Prompt starting of empirical antimicrobials based on the local susceptibility pattern, followed by modification of treatment in accordance with the antimicrobial susceptibility report which can significantly reduce the morbidity and the mortality associated with IAI.

**AUTHOR CONTRIBUTION**

Concept and design of the study were done by the first, second, and fourth author. Data collection, Data Analysis and manuscript writing were done by first, second, and corresponding author.

**CONFLICT OF INTEREST**

Authors declare no conflicts of interests.

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Self.

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