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BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL RESISTANCE PATTERN OF INTRA ABDOMINAL INFECTIONS IN A TERTIARY CARE CENTRE

Microbiology

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ABSTRACT

Background and Objectives: Bacterial isolates from Intra-abdominal infections, in particular, peritonitis and their unpredictable antimicrobial resistance patterns, continue to be a matter of concern not only globally but regionally too. An attempt in the present study was made to study the patterns of drug resistance in bacterial isolates, especially gram negative bacilli in intra-abdominal infections (IAI) in a tertiary care centre. **Materials and Methods:** From 100 cases of peritonitis, identification of isolates was done as per recommended methods. Antimicrobial susceptibility and extended-spectrum Beta-lactamase (ESBL) testing were performed following the CLSI guidelines. Results: In this study, the most common pathology was acute appendicitis which accounted for 49%.Peritonitis accounted to about 35% followed by Intra abdominal abscess with 9 % and Pelvic abscess with 4%.Out of these 100 cases, 32 (32%) cases were culture positive and 68 (68%) cases were Culture negative. Of the 32 culture-positive cases, Escherichia coli was isolated from 7 (21.9%) cases followed by Proteus vulgaris 6 (18.8%), Proteus mirabilis 5 (15.6%),Klebsiella oxytoca 5 (15.6%)and Klebsiella pneumoniae 4(12.5%).The overall prevalence rate of ESBL was 31.25%.Of which 2 (20%) isolateswereESBLpositiveandProteusspp.5(50%)wereESBLpositive.Among Klebsiella isolates, 30 % (n = 3) were ESBL positive. Tigecycline and Colistin appeared as the most active drugs against ESBL E.coli and Klebsiella spp.Conclusion:Knowing the prevalent pattern of antimicrobial resistance is an important issue especially when gram negative isolates continue to exhibit rampant resistance to various currently in use antimicrobial agents. Therefore, empirical selection of the right kind of anti-microbial therapy is significant in reducing morbidity and mortality.

KEYWORDS

Intra-Abdominal Infections (IAI), Bacterial isolates, *E.coli* , ESBL

Introduction

Intra abdominal infections (IAIs), one of the most common complications after surgery, occur in the abdominal cavity, the retro peritoneum and the abdominal organs. IAIs include acute cholecystitis and Biliary tract infection, bacterial liver abscess, acute peritonitis, and acute pancreatitis with secondary bacterial infections.

Intra abdominal infections are generally the result of invasion and multiplication of enteric bacteria in the wall of a hollow viscous or beyond. Escherichia coli is the most common organism isolated from the patients who have intra-abdominal pathology.(1)

The Microbiology of intra-abdominal pathologies is significantly altered in patients who have been exposed to the health care setting. This alteration may be due to the acquisition of Nosocomial pathogens or may reflect prior antimicrobial therapy that has selected for resistant organisms. Appendicular pathology is the most common intra-abdominal pathology followed by colonic pathologies, duodenal pathologies and Biliary pathologies.(2)

Early adequate source control remains the cornerstone of intra-abdominal infection management. Early control of the septic source can be achieved either by non-operative or operative means. Timing and adequacy of source control is the most important issue in the management of intra-abdominal infections, because an inadequate and late operation may have a negative effect on outcome.(3,4)

Concomitant adequate empiric antimicrobial therapy further influences patients morbidity and mortality. Inappropriate antibiotic therapy of intra-abdominal infections may result in poor patient outcome and the selection of an appropriate agent is a real challenge because of the emerging resistance of target organisms to commonly prescribed antibiotics.

Drug resistance continues to be a major concern in isolates from intra-abdominal infections. Treatment with appropriate antibiotics preceded by antimicrobial resistance testing aided by early diagnosis, adequate surgical management, and knowledge of antibiotic resistant organisms appears effective in reducing morbidity and mortality in IAI cases. Hence it is essential to study the bacterial profile, antibiotic Sensitivity and resistance pattern of organisms in Intra abdominal infections in a tertiary hospital.

Materials and Methods

This prospective study was conducted Department of Microbiology and Department of Surgery at Tirunelveli Medical College Hospital, Tirunelveli, and Tamil Nadu August' 2016 and September' 2016.The study protocol was approved by the Institutional Scientific and Ethics Committee. 100 Clinical specimens (Peritoneal fluid,Bile,Ascitic fluid, Pus/exudates, 0.5 ml minimum) were collected from patients undergoing major elective and urgent/emergency abdominal procedures for Appendicitis, Perforation, peritonitis, Cholilithiasis, Choledocolithiasis: malignancies, trauma.

The majority of specimens were obtained during surgery, though some specimens were also taken post-operatively from cases of burst abdomen. The specimens were processed for isolation of aerobic and anaerobic bacteria by using standard microbiological techniques and their antimicrobial susceptibility was determined by using Kirby-Bauer disk diffusion technique recommended by Clinical & Laboratory Standards Institute (CLSI), 2010 guidelines.(5)For antimicrobial susceptibility testing quality control was performed using *Escherichia coli* ATCC 25922;*Klebsiella pneumoniae* ATCC 700603 Extended Spectrum Beta-Lactamase (ESBL) positive control strain and *Pseudomonas aeruginosa* ATCC25843 strains.

ESBL detection

Screening test for ESBLs

According to the CLSI guidelines, isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 µg), ≤ 25 mm with Ceftriaxone (30 µg), and ≤ 27 mm with Cefotaxime (30 µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production.

Confirmatory tests for ESBLs The following two procedures were carried out in the present study as per CLSI guidelines:(5)

Phenotypic confirmatory test with combination disk:
This test requires the use of a third-generation cephalosporin antibiotic disk alone and in combination with clavulanic acid. In this study, a disk of Ceftazidime (30µg) alone and a disk of Ceftazidime + Clavulanic acid (30 µg/10 µg) were used. Both the disks were placed at least 25 mm apart, centre to centre, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured.

Interpretation: When there is an increase of ≥ 5 mm in inhibition zone diameter around combination disk of Ceftazidime + Clavulanic acid versus the inhibition zone diameter around Ceftazidime disk alone, it confirms ESBL production.

MIC reduction test:
The isolates positive with combination disk test were further confirmed for ESBL production by this test. Minimum inhibitory concentration of the isolates was determined by E test method. (Himedia-Ezy MIC strips) The values of range of concentration of antibiotics tested were as follows:

Ceftazidime: 0.5 µg/ml to 32 µg/ml
Ceftazidime-clavulanic acid: 0.064 µg/ml to 4 µg/ml

Interpretation
ESBL positive strain – When the ratio of the value obtained for Ceftazidime to the value of Ceftazidime in combination with Ceftazidime-clavulanic acid is more than 8.

ESBL negative strain - When the ratio of the value obtained for Ceftazidime to the value of Ceftazidime in combination with Ceftazidime-clavulanic acid is less than 8 or equal. Control strain used to validate susceptibility tests was E. coli ATCC 25922.

Statistical analysis
Data from this study was analyzed descriptively using SPSS 22.0 software. One–way analysis of variance (ANOVA) was used to determine significant difference where (P≤0.05) is significant and (P≥0.05) is not significant.

Results
A total of 100 cases of intra-abdominal infections were studied in Department of Microbiology and General Surgery at Tirunelveli Medical College hospital from August'2016 and September'2016(2 months).Out of these 100 cases, 80 (80%) cases were Males and 20 (20%) cases were Females. Most of cases was in the age group of 41-60 years and above followed by that of 21-40 years.

A total of 100 clinical specimens (Pus, Bile, Ascitic fluid, Peritoneal fluid) from 100 cases of intra-abdominal infections were collected aseptically from patients presenting with clinical picture of Intra-abdominal infections and subjected to culture and isolation for clinically significant isolates.

Incidence of Intra Abdominal infections in the study group was depicted in **Table-1.**

Table 1: Incidence of Intra Abdominal infections in the study grouzp

Sl. no	Disease	Percentage
1	Appendicitis	49%
2	Peritonitis	35%
3	Intra abdominal abscess	9%
4	Pelvic abscess	4%
5	Perforation injuries	1%
6	Cholilithiasis	1%
7	Choledocolithiasis	1%

Out of these 100 cases, 32 (32%) cases were culture positive and 68 (68%) cases were Culture negative. Of the 32 culture-positive cases, Escherichia coli was isolated from 7 (21.9%) samples; *Proteus vulgaris* was isolated from 6 (18.8%) samples; *Proteus mirabilis* 5 (15.6%) samples; *Klebsiella oxytoca* was isolated from 5 (15.6%) samples; *Klebsiella pneumoniae* was isolated from 4 (12.5%) samples; *Pseudomonas aeruginosa* was isolated from 3 (9.4%) samples and *Staphylococcus aureus* was isolated from 2 (6.3%) samples.

(Table-2)
Table2: Organism Wise Distribution Of Samples

Organism	No of growth	Percentage
Escherichia coli	7	21.9%
Proteus vulgaris	6	18.8%
Proteus mirabilis	5	15.6%
Klebsiella oxytoca	5	15.6%
Klebsiella pneumoniae	4	12.5%
Pseudomonas aeruginosa	3	9.4%
Staphylococcus aureus	2	6.3%

The overall prevalence rate of ESBL was 31.25%.The most commonly isolated organism was E. coli, of which 2 (20%) isolates and Proteus spp. 50 % (n = 5) were ESBL positive. Among Klebsiella isolates, 30 % (n = 3) were ESBL positive.(Table-3)

Table-3 Frequency of ESBL Production in Gram negative isolates

Sl. no	Organism	Percentage
1	*Escherichia coli*	20%
2	*Klebsiella spp.*	30%
3	*Proteus spp.*	50%

Table 4 shows the antibiotic susceptibility of the bacterial isolates from culture-positive samples.

Table 4: Antimicrobial resistance Pattern of Isolates

Organism	Amikacin	Gentamicin	Ciprofloxacin	Septran	Ceftazidime	Cefotaxime	Ceftriaxone
E.coli (n=7)	S-29%	S-14%	S-0%	S-0%	S-0%	S-0%	S-0%
	R-71%	R-86%	R-100%	R-100%	R-100%	R-100%	R-100%
P.vulgaris (n=6)	S-33%	S-17%	S-83%	S-0%	S-0%	S-0%	S-0%
	R-67%	R-83%	R-17%	R-100%	R-100%	R-100%	R-100%
P. mirabilis (n=5)	S-20%	S-20%	S-20%	S-0%	S-0%	S-0%	S-0%
	R-80%	R-80%	R-80%	R-100%	R-100%	R-100%	R-100%
Kleb pneumoniae (n=5)	S-0%	S-0%	S-0%	S-0%	S-0%	S-0%	S-0%
	R=100 %	R=100 %	R-100%	R100%	R-100%	R-100%	R-100%
Kleb oxytoca (n=4)	S-20%	S-20%	S-0%	S-0%	S-0%	S-0%	S-0%
	R-80%	R-80%	R-100%	R100%	R-100%	R-100%	R-100%
Pseudomonas (n=3)	S-33%	S-33%	S-33%	S-33%	S-33%	S-33%	S-33%
	R-67%	R-100%	R-100%	R-67%	R-67%	R-67%	R-67%

Organism	Amikacin	Gentamicin	Ciprofloxacin	Clindamycin	Erythromycin	Cefotaxime	Ceftriaxone
Staph. aureus (n=2)	S-0%	S-50%	S-50%	S-50%	S-50%	S-0%	S-0%
	R-100%	R-50%	R-50%	R-50%	R-50%	R-100%	R-100%

Table 5 shows the antibiotic resistance of the bacterial isolates from culture-positive samples.

Table 5: Antimicrobial resistance Pattern of Gram Negative Isolates

Organism	Imipenem	Meropenem	Cefoperazone+ sulbactam	Piperacillin+ Tazobactam	Colistin	Tigecycline
E.coli (n=7)	S-43%	S-14%	S-15%	S-15%	S-100%	S-100%
	R-57%	R-86%	R-85%	R-85%	R-0%	R-0%
P.vulgaris (n=6)	S-66%	S-0%	S-83%	S-83%	S-100%	S-100%
	R-34%	R-100%	R-17%	R-17%	R-0%	R-0%
P. mirabilis (n=5)	S-80%	S-80%	S-60%	S-80%	S-100%	S-100%
	R-20%	R-20%	R-40%	R-20%	R-0%	R-0%
Kleb pneumoniae (n=5)	S-75%	S-75%	S-75%	S-75%	S-100%	S-100%
	R-25%	R-25%	R-25%	R-25%	R-0%	R-0%

Kleb oxytoca (n=4)	S-40%	S-40%	S-40%	S-80%	S-100%	S-100%
	R-60%	R-60%	R-60%	R-20%	R-0%	R-0%
Pseudomonas (n=3)	S-66%	S-66%	S-66%	S-66%	S-100%	S-100%
	R-34%	R-34%	R-34%	R-34%	R-0%	R-0%

Discussion

Intra-abdominal infections (IAIs) represent one of the most common clinical problems in hospital practice, especially in surgical areas and centres of intensive care. The treatment of IAIs generally involves the draining of abscesses and empirical antimicrobial therapy. Bacterial isolates from intra-abdominal infections and their unpredictable antimicrobial resistance patterns, continue to be a matter of concern not only globally but regionally too.

An attempt in the present study was made to study the patterns of drug resistance in bacterial isolates, especially gram negative bacilli in intra-abdominal infections (IAI) in a tertiary care hospital. From 100 cases of Intra-Abdominal Infections(IAI), identification of isolates was done as per recommended methods. Antimicrobial susceptibility and extended-spectrum beta-lactamase (ESBL) testing were performed following the CLSI guidelines.

Out of these 100 cases, 80 (80%) cases were Males and 20 (20%) cases were Females. Most of cases was in the age group of 41-60 years and above followed by that of 21-40 years. In this study, the most common pathology was acute appendicitis which accounted for 49%.Peritonitis accounted to about 35% followed by Intra abdominal abscess with 9 % and Pelvic abscess with 4%. Cholilithiasis, Choledocolithiasis were present in 1% of cases and Perforation injury in 1% of cases. Similar findings seen in a study by K. Krobot et al., it was found that appendicular pathology was the most common intra-abdominal pathology with 38%, colonic pathologies accounted with 26%, duodenal pathologies with 23% and Biliary pathologies for which cholecystectomy was done accounted to about 6%.(6)

In a different study by Sartelli et al.,the most common intra-abdominal infection was Appendicitis. It accounted to about 38.4% of cases for which appendicectomy was done. Cholecystitis was seen in 14.4% of cases for which cholecystectomy surgery was done. Gastro duodenal suturing was done for gastro duodenal perforation which accounted for 8.1%. Other common pathologies accounted in 4.9% of cases.(7)

Out of these 100 cases, 32 (Of the 32 culture-positive cases, *Escherichia coli* was isolated from 7 (21.9%) samples; *Proteus vulgaris* was isolated from 6 (18.8%) samples; *Proteus mirabilis* 5 (15.6%) samples*; Klebsiella oxytoca* was isolated from 5 (15.6%) samples; *Klebsiella pneumoniae* was isolated from 4 (12.5%) samples; *Pseudomonas aeruginosa* was isolated from 3 (9.4%) samples and *Staphylococcus aureus* was isolated from 2 (6.3%) samples. In this study, the most common pathogens were identified to be Gram-negative bacteria (93.7%) in accord with the several global and regional reports.

This study also revealed E. coli as the most frequent pathogen associated with IAI.(8,9) As also reported by others, in addition to *E. coli, Klebsiella spp,Proteus* spp. *Pseudomonas. aeruginosa* were among other gram negative isolates.(10-12) In a study by Stephen P. Hawser in 2008,(29) from several hospitals in India (SMART study), the most frequently isolated pathogens were *E. coli* (62.7 %), *K. pneumoniae* (16.7 %), and *Pseudomonas. aeruginosa* (5.3 %) while in another similar study by Maria Virginia Villegas et al. from 10 Latin American countries*, E. coli* and *K. pneumoniae* were the most commonly isolated organisms from such IAI.(11)

The prevalence of multi-drug-resistant gram negative bacilli, especially ESBL producers, has increased worldwide with marked regional variations of their distribution.(10-12) In the present study, ESBL producers were seen to the extent of 20% in *E. coli* and 30% in *Klebsiella spp.*and 50% in *Proteus spp*. Stephen P. Hawser et al. reported 67 % and 55 % ESBL production respectively in *E. coli and K. pneumoniae* isolates in a SMART study from India.(13)

Apart from SMART global surveillance programme, several other local reports have also described variable ESBL rates in India, for example, Aggarwal et al.(14) reported ESBL rates as 22 % in E. coli and *Klebsiella pneumoniae* in Pune. Frequency of ESBL producers as low as 26% and 35% respectively in *E. coli and Klebsiella spp*. has been observed in Latin American countries,(11) while the same has been reported as 36.8% and 26.3% in *E. coli* and *Klebsiella spp.* in Asia-Pacific region.(9) Variations in ESBL rates as documented by these studies reinforce the prevalence of regional variations and emphasize the need for frequent studies.

For antimicrobial resistance, interestingly, in *E. coli*, resistance was as high as 100% for ciprofloxacin, 100% for Third generation cephalosporins and also was relatively high forPiperacillin/Tazobact am (85 %) and Cefoperazone/Sulbactam (85%) combinations. Among Amino glycosides, resistance levels of 71% for Amikacin, 86% for Gentamicin, while 57% and 86% for Carbapenems i.e. Imipenem and Meropenem respectively were evident. The resistance level for Ciprofloxacin,Aminoglycosides and Carbapenems were comparati vely higher than that reported by other investigators such as by Sepehri G, Goldstein and Syndman and Paterson et al. These investigators reported such resistance in the range 3-23%.(15-17)

For antimicrobial resistance, interestingly, in Proteus spp., resistance was as high as 100% for ciprofloxacin, 100% for Third generation cephalosporins and was relatively low for Piperacillin/Tazobactam (17 %) and Cefoperazone/Sulbactam (17%) combinations. Among Amino glycosides, resistance levels of 67% for Amikacin, 83% for Gentamicin, while 20% and 34% for Carbapenems i.e. Imipenem and Meropenem respectively were evident. The resistance level for Ciprofloxacin,Aminoglycosides and Carbapenems were comparativel y higher than that reported by Thomas et al.(18)

Klebsiella spp. was relatively more resistant to commonly used antimicrobial agents with resistance levels as high as 100% for Third generation cephalosporins, Ciprofloxacin and also other antimicr obials Amikacin Gentamicin.25% for Piperacillin /Tazobact am combination, 25% for Cefoperazone/ sulbactam, 25% for Carbapenems i.e. Imipenem and Meropenem were observed. These results are in accord with several other studies that revealed an increase in resistance in Klebsiella spp. from different regions of the world, namely Chen et al., Hauser et al., Sepehri and Villages et al.(9,11,12)

Tigecycline and Colistin appeared as the most active drugs against E. coli and Klebsiella spp.(100% susceptible) which again tallies with the results of other investigators, namely Villages et al., Hauser et al., Chaudhuri et al.(11,12,17)Genotypic resistance marker analyses are limitations of this study but clearly increase in resistance, whether due mainly to ESBL production or hitherto other yet unclear mechanisms with tagged clinical impact remain a potential threat in treating such infections.(11) Plasmids responsible for ESBL production carrying genes encoding resistance to several other structural drug classes' especially Amino glycosides and Fluoroquinolones have been identified making options of treating such infections difficult and extremely limited. In addition, fast emerging reports of Carbapenem-resistant isolates are making situation still worse.(16)

Pseudomonas aeruginosa is the other major Nosocomial pathogen which displays remarkable properties of resistance to many antibiotics; therefore, their isolation is an important cause of concern.(19-21)Three strains of Pseudomonas aeruginosa showed resistance level of 67% for ciprofloxacin, Third generation cephalosporins, Amikacin and Gentamicin. and was relatively low for Piperacillin/Tazobactam (34 %) and Cefoperazone/Sulbactam (34%) combinations and 34% for Carbapenems i.e. Imipenem and Meropenem while Colistin and Tigecycline were highly effective It is comparatively lesser when compared with other studies.(19-21) The present study did not include screening of Amp C beta lactamases and Metallo beta lactamases (MBLs) production which is an important limitation of our study.

Among gram positive isolates, the number of isolates is too less to make any definitive conclusions. Only 2 strains of *Staphylococcus aureus* isolated in the present study revealed resistance level of 50% toward Gentamicin ,Erythromycin, Clindamycin, and Ciprofloxacin and 100% towards Amikacin.

In this study, commonly used antimicrobials showed the lowest antibacterial activity against microbial isolates from Intra Abdominal infections. The high antimicrobial resistance to commonly used antimicrobials could profoundly affect the choice of therapeutic agents. So the choice of antimicrobial therapy must be based on culture

growth and antibacterial sensitivity tests and also take into account the risk of inadequate and inappropriate antimicrobial therapy and emergence of bacterial resistance to commonly used antimicrobials.

Conclusion

Knowing the prevalent pattern of antimicrobial resistance is an important issue especially when gram negative isolates continue to exhibit rampant resistance to various currently in use antimicrobial agents. Therefore, empirical selection of the right kind of antimicrobial therapy is significant in reducing morbidity and mortality. In Indian context, data especially antimicrobial resistance status is still scarce for this very common problem.

The study emphasizes need for antimicrobial susceptibility testing of clinically significant isolates not only as a routine procedure but also on periodic basis, especially when no definitive resistance or susceptibility patterns are available in a given geographic location.

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