ORIGINAL ARTICLE

ANTIMICROBIAL SUSCEPTIBILITY OF INTRA-ABDOMINAL INFECTION ISOLATES FROM A TERTIARY CARE HOSPITAL IN KARACHI

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Background: Intra-abdominal infections are associated with significant morbidity and mortality. The most frequent pathogens involved are the gastrointestinal flora which can cause poly-microbial infections. Microbiological diagnosis is required to determine the aetiology and antimicrobial susceptibility of the organisms involved. Prompt initiation of antimicrobials is essential for improving patient's outcome. Knowledge of local trends of antimicrobial resistance in nosocomial isolates is essential for empiric therapy. **Methods:** A total of 190 clinical isolates collected from intra-abdominal infections during July 2013 to July 2014 were included in the study. Organism identification and Antimicrobial sensitivity testing using standard biochemical tests and CLSI recommended criteria was carried out. **Result:** Of the total 190 isolates from abdominal infection sources 52% were from fluid sources (peritoneal & ascitic fluid), 41% were from gall bladder and 6.5% were from other abdominal sources. *E. coli* (46.8%) was the most frequently isolated gram negative and *Enterococcus* (13.1%) was the most frequently isolated gram positive organism. Carbapenem (imipenem) was the most active agent against enterobacteraceae exhibiting, 94.4% and 91.3% sensitivity against *E. coli* and *Klebsiella* respectively. While vancomycin was the most active agent against gram positive organisms. Eighty-four percent of the *Enterococci* isolated were sensitive to vancomycin. Most isolates exhibited resistance to one or more antibiotics. **Conclusion:** Continuous evolution of antimicrobial resistance patterns in bacteria necessitates updating of local data on antimicrobial susceptibility profiles to ensure the safety and efficacy of pathogen specific antimicrobial therapies.

Keywords: Intra-abdominal infections (IAI), antimicrobials; resistant; sensitive
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INTRODUCTION

Intra-abdominal infections include a wide spectrum of pathological conditions that involve infectious lesions of all the intra and retroperitoneal organs. Clinically, these are divided into Uncomplicated and complicated infections. Uncomplicated are those infections where the infectious process remains limited to a single organ. In the event of a complicated infection the process extends beyond an affected organ and causes localized or diffused peritonitis.[1] These infections are associated with significant morbidity and mortality.[2] The most frequent pathogens involved are the gastrointestinal flora which can cause polymicrobial infections. Microbiological diagnosis is essential to determine the aetiology and antimicrobial susceptibility of the organisms involved.[3] Antimicrobial therapy plays an integral role in the management of these infections. Prompt initiation of antimicrobials is essential for improving patient outcome.[4] Delays in diagnosis and the use of inadequate therapies for the treatment of intra-abdominal infections can result in increased therapy failures and high mortality rates.[5] Empiric antimicrobials active against gram negative organisms in case of community acquired IAI and additional coverage for *Enterococci* and MRSA in case of HAI is recommended. Infectious Disease Society of America recommends the use of ampicillen-sulbactam, ertapenem, imipenem, meropenem, piperacillin–tazobactam, cephalosporins used in combination therapy and quinolones in combination with metronidazole. Once the microbial identification and sensitivity is available the therapy should be tailored accordingly.[6] Unnecessary use of broad spectrum antibiotic therapy may result in the acquisition of intrinsically drug resistant organisms and selection of resistance within the hospital.[7]

Microbial resistance and the available antimicrobials vary from region to region. Local trends of antimicrobial resistance in nosocomial isolates should therefore, dictate the empiric therapy.[8] Management guidelines for complicated intra-abdominal infections are written for the Western context.[6,9] As few Asian surveys are available, the clinicians have to rely on the international data available. Although, our study was a single centre study from a Tertiary care hospital in Pakistan, but provides microbial profile and antimicrobial susceptibility pattern in an Asian setting. The information from such studies can be helpful in prescribing appropriate empirical therapy and developing antimicrobial stewardship to prolong the utility of available antibiotics.

MATERIAL AND METHODS

The study was carried out at the Department of Microbiology, Liaquat National Hospital, Karachi. Approval for the use of clinical data for this study was

obtained from the Ethical Review committee of the Hospital. All clinical isolates derived from Intra-abdominal samples received from July 2013 to July 2014 were included in the study. Only one isolate per patient was accepted. Aerobic and facultative bacteria were cultured from specimens from intra-abdominal body sites, e.g. appendix, peritoneum, colon, bile, pelvis and pancreas. Isolates from any other extra-abdominal sources were excluded. Organisms isolated anaerobically were also excluded from the study.

The samples were inoculated on blood, chocolate and MacConkey agar. After incubation at 35 ºC for 24–48 hrs, if a bacterial growth was obtained then further identification of the organism was carried out using standard biochemical tests.

The biochemical tests for gram negative organisms included carbohydrate fermentation tests, carbon utilization tests, Indole test, Urease test, Hydrogen sulphide production test and oxidase test. Further confirmation where required was done using commercially available API strips 20 E and 20 NE.

Tests were also carried out for the final identification of gram positive bacteria. These included type of haemolysis, Nacl 6.5% tolerance, Catalase test, coagulase test. Bile esculine test. Susceptibility to Bacitracin and optochin. Commercially tested API strips were used if required for the final identification of the organism. Antimicrobial susceptibility testing was performed according to Clinical Laboratory Standards Institute guidelines (CLSI). Antibiotic sensitivities of all isolated organisms was carried out using the Kirby Bauer (disc diffusion) method as recommended by CLSI. An inocculum corresponding to 0.5 McFarland standard was used. E-test for MIC strips were used where recommended, using the CLSI criteria. Appropriate control strains were used to ensure the validity of the results. Susceptibility patterns were noted. The data obtained during the study was statistically analysed using computer package SPSS version 16.

RESULTS

Overall, 190 clinical isolates were collected from intra-abdominal samples between July 2013 to July 2014. These consisted of both gram negative (144 out of 190) and gram positive (46 out of 190) organisms. Identification of the isolates was done according to the standard biochemical tests. Of the total isolates 41% (78 out of 190) were from gall bladder while 52% (99 out of 190 of all isolates) were from fluid sources (peritoneal & ascetic fluid). The remaining 6.5% (12 out of 190 isolates) were from various other sources. Majority of the isolates from all clinical specimen were gram negatives 76% (144 out of 190 isolates) while gram positive organisms were 24% (46 out of 190 isolates).

The most frequently isolated species was *E.coli* 46.8% (89 out of 190 isolates), followed by *Enterococcus* 13.1% (25 out of 190), *Klebsiella spp* 12.1% (23 out of 190 isolates), *S.milleri* 7.3% (14 out of 190 isolates) *Pseudomonas spp* 5.7% (11 out of 190 isolates) , *Acinetobacter spp* 4.2% (8 out of 190 isolates), *S. aureus* 3.5% (7 out of 190 isolates), *P. aeruginosa* 3.1% (6 out of 190), *Stenotrophomonas spp* 2.1% (4 out of 190) and *Enterobacter spp* 1.5% (3 out of 190 isolates).

Carbapenem (imipenem) was the most active agent against enterobacteraceae exhibiting, 94.4% and 91.3% sensitivity against *E.coli* and *Klebsiella* respectively. However, only 66.7% of *P.aeruginosa* were sensitive to imipenem. Polymyxin B was the only antibiotic which showed consistent activity (100%) against *P. aeruginosa* and *Acinetobacter spp.*

The gram positive organisms isolated were *Enterococci, S. aureus* and *streptococcus spp.* Only 28% of the *Enterococci* were sensitive to ampicillin, while 84% were sensitive to vancomycin. Vancomycin was also the drug showing consistent activity against *S. aureus* and *S. milleri* isolated (100%).

Table-1: Percentages of Gram negative intra-abdominal isolates susceptible to the antimicrobial agents used in the study.

	E.Coli	*Kleb. spp*	*Ps. spp*	*P.aeru*	*Aci. spp*	*Steno.spp*	*Enterob spp*
No.	89	23	11	6	8	4	3
AMC	23.6	47.8	NT	NT	0	NT	0
CRO	32.6	43.5	NT	NT	0	NT	33.3
TZP	73.0	73.9	81.8	66.7	0	NT	33.3
IPM	94.4	91.3	100	66.7	0	NT	100.0
CAZ	NT	NT	81.8	50.0	0	75.0	NT
SCF	77.5	60.9	NT	NT	12.5	NT	NT
TOB	NT	NT	27.3	66.7	37.5	NT	NT
CN	75.3	69.6	37.3	66.7	0	NT	100.0
AK	91.0	82.6	27.3	83.3	0	NT	100.0
CIP	42.7	52.2	54.5	50	0	75	100.0
LEV	NT	NT	57.1	NT	NT	100	NT
SXT	38.2	65.2	NT	50	25.0	100	100.0
PB	100.0	100.0	100.0	100.0	100.0	50	100.0

NT=Not Tested, Kleb.spp=Klebsiella spp, Ps spp=pseudomonas spp, P.aerug=P.aeruginosa, Aci. spp=Acinetobacter spp, Steno. Spp=Stenotrophomonas spp, Enterobac spp=Enterobacter spp, AMC=amoxi-clavulanate, CRO=ceftriaxone, TZP=piperacilli-tazobactam, IPM=imipenem, CAZ=ceftazidime, SCF=cefoperozone-sulbactam, TOB=tobramycin, CN=gentamicin, AK=amikacin, CIP=ciprofloxacin, LEV=levofloxacin, SXT=cotrimaxozole, PB=polymyxin

Table-2: Percentages of Gram positive intra-abdominal isolates susceptible to the antimicrobial agents used in the study

	Enterococcus	*S.milleri*	*S.aureus*
No.	25	14	7
FOX	NT	NT	28.6
AMP	24.0	100.0	NT
AMC	28.0	100.0	NT
CRO	NT	100.0	NT
VA	84.0	100.0	100.0
AK	NT	NT	42.9
E	NT	50.0	28.6
DA	NT	NT	42.9
FD	NT	NT	71.4
MH	NT	NT	100.0
SXT	NT	NT	57.1
C	80.0	92.9	NT
OT	20.0	100.0	NT
LZD	100.0	NT	NT

NT=Not Tested, FOX=cefoxitin, AMP=ampicillin, AMC=amoxi-clavulanate, CRO=ceftriaxone, VA=Vancomycin, AK=amikacin, E=Erythromycin, DA=Clindamycine, MH=minocycline, C=chloremphenicol, OT=Tetracycline, ZD=Linezolid

DISCUSSION

The intra - abdominal pathogens isolated during our study consisted mostly gram negative organisms (*E. coli,* and *Klebsiella*). Studies carried out in India also show gastrointestinal flora to be the most frequent pathogens with *E.coli* (62.7%) being the most frequently isolated organism in one of the studies.[8,10] A multicenter CIAO study carried out in 66 European medical institutes also found *Enterobacteriacaea* to be the main pathogens involved in intra-abdominal infections.[4] Empiric coverage against these organisms should be provided and once antimicrobial sensitivity report is available the therapy can be modified accordingly. Majority of the isolates in our study originated from gall bladder and peritoneal fluid, though other sources in a much smaller number were also represented. Antibiotic susceptibility results showed that the majority of species demonstrated resistance to multiple antibiotics. There was a high rate of resistance to third generation Cephalosporins (ceftrioxone, cefotaxime, ceftazidime) and Quinolones. (Table-1)

This finding is consistent with other studies showing nosocomial infections caused by gram negative organisms resistant to third generation cephalosporins.[11] Resistance to third generation cephalosporins (ceftazidime, cefotaxime) is considered as potential marker for the presence of Extended Spectrum β lactamases (ESBL).[12]

Quinolone resistance (ciprofloxacin) was high among both gram positive and gram negative organisms isolated in our study. Although, according to other studies quinolones are still quite active against most *enterobacteriaceae* and non-fermentative gram negative bacilli with the exception of pseudomonas.[13] Carbapenem (imipenem) was consistently the most active agent against gram negative organisms with the exception of *P. aeruginosa* strains for which it showed susceptibility of 66%, as observed by other studies also.[6]

Among the Hospitalized patients use of broad spectrum antibiotics as empirical therapy can be a cause of infection with gram negative organisms like *B. cepacia* which are intrinsically resistant to most empirically used antibiotics such as aminoglycosides, first and second generation cephalosporins and polymyxins. Thus, limiting the therapeutic options.[14,15] Adherence to strict infection control policies is required to limit the spread of multiple drug resistant organisms.

Addition of empiric gram positive coverage is recommended for the treatment of Hospital acquired intra- abdominal infections. Only 28% of *Enterococci* were sensitive to ampicillin, the recommended first line therapeutic agent.[16] *Enterococci* showed 84% sensitivity to vancomycin while *staphylococci* were 100% sensitive. Isolates showing resistance to methicillin were also found to be sensitive to vancomycin. Studies from Egypt and India report the emergence of vancomycin-intermediate and resistant *S aureus* (VISA, VRSA). All of them were also methicillin resistant.[17,18] The *S. aureus* isolated in our study, both MRSA and MSSA were 100% sensitive to vancomycin with MIC <0.5 µg/ml. Vancomycin was the most effective drug in the treatment of gram positive infections in our study. (Table-2)

Due to continuous evolution of antimicrobial resistance in bacteria, local antimicrobial spectrum should be taken into consideration when initiating empirical therapy. To improve the treatment outcome of nosocomial infections, antimicrobial restriction policies might help in limiting the emergence of resistant organisms. In addition, a comprehensive policy for infection

control in hospitals must be designed and implemented to decrease the risk of nosocomial infections. It may also be helpful to monitor our environment, improve the sanitary and water distribution systems and control the indiscriminate usage of antimicrobial agents.

CONCLUSION

The continuous evolution of antimicrobial resistance patterns in bacteria necessitates continuous updating of data on antimicrobial susceptibility profiles to ensure the safety and efficacy of pathogen specific antimicrobial therapies. More data from such studies in Asian countries will help the physicians to select antibiotic therapies for IAI that are appropriate and specific for their location.

AUTHORS' CONTRIBUTION

US, SA: Conceptualization of study design, data interpretation, data analysis and proof reading. UZK, MS, HS: Data collection & analysis, proof reading.

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