

Antimicrobial resistant gram negative isolates from blood stream infections: A report from BPKIHS

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Abstract

Objective: To find out resistance pattern and production of ESBL among gram negative blood culture isolates.

Methods: This study was carried out in Microbiology laboratory, BP Koirala Institute of Health Sciences, Dharan, Nepal from July 2011-June 2012. Altogether 150 gram negative bacteria isolated from cases of blood stream infections were evaluated. Isolation, identification of bacteria, determination of antimicrobial susceptibility pattern and detection of ESBL were done following standard protocol.

Results: Resistance to commonly used antimicrobials were noted in varying frequencies. Most of the isolates were resistant to ampicillin (97%) and sensitive to imipenem (98%). Forty seven percent of isolates were ESBL producers and a substantial number of isolates were resistant to at least 3 routinely used drugs.

Conclusion: The study concluded that high resistance was found among the blood culture isolates and ESBL production was seen in a significant number of isolates.

Keywords: Antimicrobial resistance, Blood culture, Gram Negative Bacteria, ESBL.

1. Introduction

Antimicrobial resistance (AMR) is a global threat which is associated with increased morbidity and mortality and it appears to be growing. Multidrug resistance (MDR) is another rising issue that further complicates the problem [1]. The bacterial isolates have developed resistance to many available drugs through various mechanisms including production of enzymes like beta lactamases, extended spectrum beta lactamase, carbapenemase etc. [2]. Extended spectrum beta lactamases (ESBL) constitute plasmid mediated enzymes of bacteria that confer resistance to the penicillins, monobactam antibiotic aztreonam and cephalosporins such as cefotaxim, ceftriaxone and ceftazidime. These enzymes are commonly produced by *Escherichia coli* and *Klebsiella pneumoniae* but also found in other species of Enterobacteriaceae as well as other gram negative organisms like *Pseudomonas* species and *Acinetobacter* species. These bacteria have spread rapidly

and become an important cause of transferable multidrug resistance [MDR] in gram negative bacteria [3]. This study was conducted with an aim to find out the antimicrobial resistance pattern of gram negative bacteria and prevalence of ESBL producing gram negative bacteria isolated from blood culture in BPKIHS.

2. Materials and methods

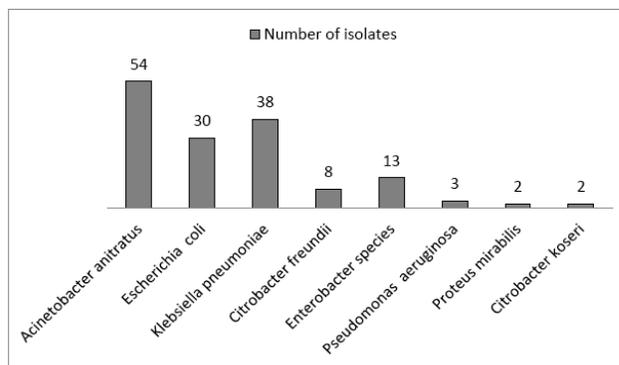
The study was carried out in Department of Microbiology, BP Koirala Institute of Health Sciences, Dharan, Nepal from July 2011 – June 2012. A total 6262 blood samples from clinically diagnosed blood stream infection cases were received from various departments during this period. Specimens at the time of receipt were already inoculated onto brain heart infusion (BHI) broth. After 24 and 48 hours of aerobic incubation, subcultures were performed in blood agar and MacConkey agar.

Growth was recorded the next day [4]. Bacterial growth if present was processed for further identification and antimicrobial susceptibility testing. Identification of bacteria was done according to the Gram's reaction and biochemical tests like catalase, oxidase, citrate, urease, sulphide indole motility, triple sugar iron and other sugar fermentation tests using conventional microbiological procedure according to standard protocols [5]. Antimicrobial susceptibility test was performed on Mueller Hinton agar by Kirby Bauer disk diffusion method, using drugs like ampicillin (10 µg), cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg), ciprofloxacin(5µg), gentamicin(10µg), imipenem(10µg) [by HiMedia laboratories, India] and results were interpreted according to CLSI guidelines[6]. All isolates were tested for ESBL production by double disk synergy test. An enhanced zone of inhibition with a diffusion of ≥5mm around the ceftazidime clavulanic acid disk as compared to ceftazidime disk alone was interpreted as presence of ESBL [7].

3. Results

A total of 6262 blood samples received from various clinical departments yielded 150 gram negative bacteria. The most common gram negative bacteria isolated from blood culture was *Acinetobacter anitratus* (n=54) followed by *K. pneumoniae* (n=38). Least common were *Proteus mirabilis* and *Citrobacter koseri* [figure 1].

Figure 1: Distribution of gram negative isolates in blood culture



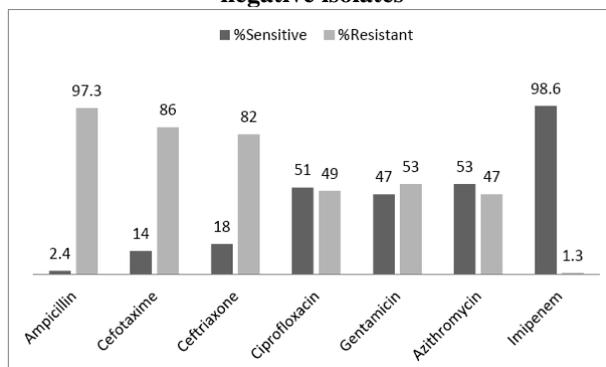
Most of the of isolates obtained from the patients belonged to the age group below 10 years (68.6%) and *Acinetobacter anitratus* was the most commonly isolated gram negative bacilli from all the age groups except for the range 31-40 years in which *Enterobacter species* was predominant (50%). *K. pneumoniae* was the second most common agent for the children <10 years, similarly *Esch. coli* was the second common agent for almost all other age groups. [Table 1]. Among the inpatients most of the organisms were isolated from department of Emergency (43%), followed by NICU (18%). Most common isolate was *A. anitratus* from almost all departments except NICU in which *K. pneumoniae* was predominant. From outpatient department most common organism isolated was *A. anitratus* (61%) followed by *K. pneumoniae* (23%).

Table 1: Distribution of organisms according to age group

Organism	0-10 years	11-20 years	21-30 years	31-40 years	41-50 years	51-60 years	>60 years	Total
<i>A.anitratus</i> (n=54)	35(64.8%)	8(14.8%)	6(11.1%)	1(1.85%)	1(1.85%)	1(1.85%)	2(3.7%)	54
<i>Esch. coli</i> (n=30)	16(53.3%)	2(6.6%)	4(13.3%)	1(3.3%)	5(16.6%)	1(3.3%)	1(3.3%)	30
<i>K. pneumoniae</i> (n=38)	30(78.9%)	2(5.2%)	3(7.8%)	0	1(2.6%)	1(2.6%)	1(2.6%)	38
<i>Enterobacter spp</i> (n=13)	9(69.2%)	1(7.6%)	1(7.6%)	2(15.3%)	0	0	0	13
<i>C.freundii</i> (n=8)	4(50%)	1(12.5%)	1(12.5%)	0	1(12.5%)	1(12.5%)	0	8
<i>C.koseri</i> (n=2)	2(100%)	0	0	0	0	0	0	2
<i>P.aeruginosa</i> (n=3)	2(66.6%)	0	0	0	1(33.3%)	0	0	3
<i>P.mirabilis</i> (n=2)	2(100%)	0	0	0	0	0	0	2
Total	100	14	15	4	9	4	4	150

Among 150 isolates resistant to commonly used drugs like ampicillin, cefotaxim and ceftriaxone was observed in 97%, 86% and 82% respectively. However, more than 98% of isolates were sensitive to imipenem by disk diffusion test [Figure 2].

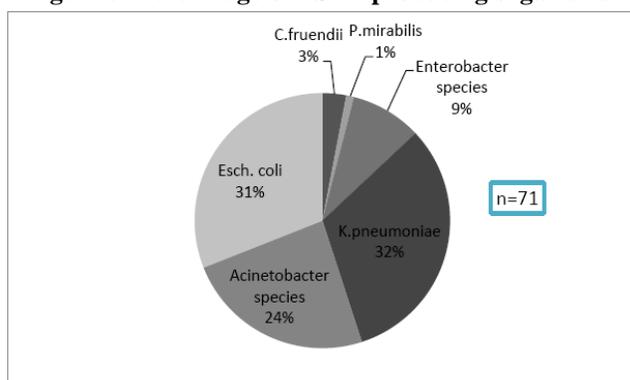
Figure 2: Antimicrobial susceptibility pattern of gram negative isolates



Regarding resistance to multidrug, around 86% of the total isolates were resistant to at least 3 antimicrobials. So, 98% *A. anitratus*, 92% *Enterobacter* species, 81%, 75% and 70% *K. pneumoniae*, *C. freundii* and *Esch. coli* respectively, were found to be resistant to ≥ 3 drugs. All the *Pseudomonas* species and *P. mirabilis* were MDR.

Among 150 organisms ESBL production was found in 71 (47.3%). *K. pneumoniae* was the most common ESBL producer. [Figure 3]

Figure 3: Percentage of ESBL producing organisms



In 22 (73%) out of 30 *Esch. coli* isolates ESBL production was found. Similarly out of 54 *A. anitratus* 17 (31.4%), out of 38 *K. pneumoniae*, 13 *Enterobacter* species and 8 *C. freundii*; 23(60.5%), 6(46.1%) and 2(25%) were found to be ESBL producers respectively. Among two *P. mirabilis* one was ESBL producer and all the *C. koseri* and *Pseudomonas* isolates did not produce ESBL.

4. Discussion

Study of bacteriological profile and antimicrobial susceptibility pattern plays an important role in effective management of bacteraemia cases [8]. In the present study the distribution of gram negative organism in blood and their resistance pattern has been evaluated. Most common gram negative organism isolated from blood was found to be *A. anitratus* (36%) followed by *K. pneumoniae* (25%) and *Esch. coli* (20%). Though, this finding is not in agreement to a study that showed most common organisms were *K. pneumoniae* 33% followed by *Esch. coli* 20% and *Acinetobacter* was isolated in 4% only [8]. But, their report was similar in finding *K. pneumoniae* predominantly in newborns as it was in the present study. Another study in India showed comparable results with the present study - *Acinetobacter* species 31% and *Esch. coli* 23% [9]. Such type of variations might be due to the different geographical locations. Antimicrobial resistance is indiscriminant, impacting every country in the world. Identifying the resistance pattern of microorganisms in every hospital is the key to success in appropriate treatment of patients [1, 10]. Findings of the present study are indicative of high resistance rates in most microorganisms. In the present IJBR (2017) 08 (09)

study almost all the isolates encountered in blood exhibited high frequency of resistance to cephalosporins like: cefotaxime (86%) and ceftriaxone (82%). Similar results were shown by Tahnkiwale *et al* from India who reported 95% of resistance to all third generation cephalosporins [11]. In the contrary, the results are higher than a previous study in our own setting which documented comparatively lower rate of resistance against cefotaxime (62%) and ceftriaxone (59%) by Shrestha *et al* [12]. From these observations it can be said that the trends for the development of resistance towards the third generation cephalosporins in our institute is in progress. Similarly, rate of resistance to ciprofloxacin and gentamicin 49% and 53% respectively were observed in the present study which is in agreement with previous study conducted in our institution [12]. It is surprising that no significant change in susceptibility pattern of gram negative bacilli to ciprofloxacin and gentamicin was observed in our hospital over last few years.

The MDR gram negative bacterial isolates possess another therapeutic challenge and infections related to these organisms have become a major problem as they are associated with increased mortality rates and higher healthcare costs [13]. Although MDR was found previously in *Acinetobacter* species and *Pseudomonas* species, now it has already extended to the members of Enterobacteriaceae family [14]. In our study the frequency of resistance to at least 3 drugs was found to be 100% in *P. aeruginosa*, 98% in *A. anitratus* and 92% in *Enterobacter* species. Similarly all other isolates demonstrated resistance towards multiple drugs. Study conducted elsewhere in Nepal show similar results [15]. Likewise, Mehdinejad *et al* from Iran obtained results showing high prevalence of MDR bacteria [8].

Most effective drug for all the gram negative isolates was found to be imipenem which is an important second line drug. It showed 98% susceptibility to all the isolates, a finding in concordance to that of Sabharwal ER [16] in India, Saghir S[17] in Pakistan and Sader HS[18] in USA which showed 100%, 96% and 99% sensitivity respectively indicating that this drug is still effective for the treatment of gram negative bacterial infections.

ESBL are the pathogens of concern nowadays as their prevalence is on the rise and they pose a great therapeutic challenge to clinical microbiologists and clinicians [19]. The prevalence of ESBL among clinical isolates varies from country to country and from institution to institution [3]. Out of 150 gram negative blood culture isolates 47% were found to be ESBL producers in present study. This result is similar to data derived from Pakistan (45%) [20], in the contrary in USA prevalence of ESBL among Enterobacteriaceae was found to be ranging from 0-25% [21].

Most frequent ESBL producing isolates in our study were *K. pneumoniae* and *Esch. coli* (32% and 31% respectively), an observation different than made by Ali *et al* which showed most frequent isolate being *Enterobacter cloacae* (79%) followed by *A. baumannii* (72%) [20].

Regarding the ESBL production in different organisms, present study demonstrated that 73% of *Esch. coli* isolates to be ESBL producers which is in concordance to the report by Mohanty S (72.3%) [22]. However it was different from other studies conducted in India which showed that the ESBL producing strains formed 39.1% and 46.5% of isolates [20,23]. Regarding *Acinetobacter* species 31% were ESBL producers which is less in comparison to the studies conducted by and Mohanty S in Northern India showing 66.79% of *A. anitratus* were ESBL producers [22]. Among the *K. pneumoniae* 60.5% showed ESBL production, a finding in agreement with that of the study by Duttatroy B (57.8%) in India [23] and Ali AM (57%) in Pakistan [20]. Among the *Enterobacter* isolates, 46.1% were ESBL producers which was slightly lower than the study conducted by Mohanty *et al* (51.2%)[22], but higher than Shah *et al*(33.3%)[24]. ESBL production in *C. freundii* was detected in only 25% of isolates of our study compared to 67.5% reported by Mohanty S [23]. Surprisingly, none of the *P. aeruginosa* isolates were found to be ESBL producers in our study, whereas Mohanty *et al* found 58.7% *P. aeruginosa* to be ESBL producers [22].

Observations made in the present study show high resistance to routinely used antimicrobials like ceftriaxone, cefotaxime, ciprofloxacin etc. although imipenem still shows good activity among bacterial isolates. MDR and ESBL production was found in significant numbers of isolates. Our study indicates that it becomes imperative to take timely steps for the detection, prevention and control of spread of such resistant organisms.

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