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# Bacteriological Profile and Antimicrobial Susceptibility Pattern among Isolates Obtained From Body Fluids

Lok Bahadur Shrestha,<sup>1</sup> Narayan Raj Bhattarai,<sup>1</sup> Basudha Khanal,<sup>1</sup><sup>1</sup>Department of Microbiology and Infectious Diseases, B. P. Koirala Institute of Health Sciences, Dharan, Sunsari, Nepal.

## ABSTRACT

**Background:** Infections of the sterile body sites, when occurs typically have greater clinical urgency and these infections could be life threatening. For the appropriate management of patient, early detection and identification of organism is crucial. Hence, the present study was designed to evaluate the prevalence, organism profile and antimicrobial susceptibility pattern of isolates obtained from infection of body fluids.

**Methods:** Laboratory and clinical records of all the sterile body fluid except blood and cerebrospinal fluid submitted to the Department of Microbiology, B. P. Koirala Institute of Health Sciences during the period of 5 years (January 2012 to December 2016) were analyzed.

**Results:** Among 1835 samples, 196 fluids samples showed growth of organisms with an isolation rate of 10.68%. *Escherichia coli* (*E. coli*) was the most common organism followed by *Staphylococcus aureus* (*S. aureus*) and *Acinetobacter calcoaceticus-baumannii* complex. Antimicrobial susceptibility testing showed variable degree of resistance. Thirty percentage of organisms were multi-drug resistant, 10% were extensively-drug resistant, none was pan-drug resistant, 35% of *E. coli* and *K. pneumoniae* were extended spectrum  $\beta$ -lactamase producer and 30% of *S. aureus* were methicillin-resistant *S. aureus*. The study showed increasing trends of multi-drug resistant, extensively-drug resistant, methicillin-resistant *S. aureus* and extended spectrum  $\beta$ -lactamase over the years.

**Conclusions:** The study showed infection rate of 10% among normally sterile body fluids. *E. coli*, *S. aureus* and *Acinetobacter calcoaceticus-baumannii* complex are the common organisms. The infections are associated with multi drug resistant organisms. Routine surveillance of multi-drug resistant in infection of body fluids is necessary to guide treatment.

**Keywords:** Extensively-drug resistant; methicillin-resistant *S. aureus*; multi-drug resistant; sterile body fluid.

## INTRODUCTION

Body fluids like pleural, ascitic, peritoneal, synovial and pericardial fluid are usually sterile. Infections of the sterile body sites, when occurs typically have greater clinical urgency and these infections could be life threatening.<sup>1,2</sup> For the appropriate management of patient, early detection and identification of organism with the results of antimicrobial susceptibility testing is crucial. Positive cultures are usually low because of less number of pathogens and prior administration of empirical antibiotics in these patients. Moreover, the emergence of antimicrobial resistance especially multi-drug resistant (MDR), extensively-drug resistant (XDR), pandrug resistant (PDR) organisms, methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum  $\beta$ -lactamase (ESBL) producers has hindered the clinical

management of the patient.<sup>3,4</sup>

Regular monitoring of bacterial susceptibility pattern a particular area is necessary for empirical treatment of infection as soon as possible, which helps in reduction of morbidity and mortality.<sup>5</sup> Hence, the current study was designed to evaluate the prevalence, organism profile and antimicrobial susceptibility pattern of isolates obtained from infection of body fluids.

## METHODS

This is a hospital based retrospective study conducted in the Department of Microbiology, BPKIHS. Ethical clearance was obtained from Institutional review board, BPKIHS. Laboratory and clinical records of all patients admitted during the period of 5 years (January 2012

**Correspondence:** Lok Bahadur Shrestha, Department of Microbiology & Infectious Diseases, B. P. Koirala Institute of Health Sciences, Dharan, Sunsari, Nepal. Email: [lok.shrestha@bпкиhs.edu](mailto:lok.shrestha@bпкиhs.edu), Phone: +9779842295909.

to December 2016) whose body fluid(except blood and cerebrospinal fluid) yielded positive growth was traced and analyzed. An attempt was made to establish the clinical significance of the isolates with clinical correlations and records of repeat culture and sensitivity.

Body fluid specimens when received in microbiology laboratory were subjected to gram staining and culture. The specimen is inoculated onto Blood agar, MacConkey agar and Chocolate agar and incubated aerobically at 35 °C for 18-24 hours. After incubation, the plates were observed for bacterial growth. Any bacterial colony was identified by using gram staining and biochemical tests following standard microbiological guidelines.<sup>6</sup>After identification, antimicrobial susceptibility testing was performed by modified Kirby Bauer disc diffusion method following clinical and laboratory standards institute guidelines.<sup>7</sup>

MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories and XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR is defined as non-susceptibility to all agents in all antimicrobial categories.<sup>8</sup>

## RESULTS

During the study period, samples from 1835 patients were submitted for culture and sensitivity. Among the patients, 54 % were male while 46% were female. Age-wise distribution of the patient showed 12% were

children, 52% were adults and 36% were elderly. Among them, samples from 196 patients (10.68%) showed growth.Total number of samples and growth rate has been further elicited in Table 1.

Table 1. Number of samples and growth rate.

Samples	Total no of samples	Culture positive	Growth percentage
Pleural fluid	1172	137	11.68
Ascitic fluid	517	42	8.12
Synovial fluid	125	15	12
Pericardial fluid	21	2	9.5
Total	1835	196	10.68

Among 137 bacterial isolates obtained from pleural fluid, *Escherichia coli* (n=34, 25%) was most common followed by *Acinetobacter calcoaceticus-baumannii* complex (ACB complex)(n=25, 18%).Similarly, *E. coli*(n=12, 28%) was also the most common organism isolated from ascitic fluid followed by *Staphylococcus aureus* (n=7, 16%). Whereas, *S. aureus* (n=7, 46%)was the most common isolate obtained from synovial fluid followed by *E. coli* (n=4, 26%). *S. aureus*(n=2) was the only bacteria isolated from pericardial fluid (Table 2).

Antimicrobial susceptibility pattern of the isolates has been tabulated in Table 3. Eighty percentage of *E. coli* were resistant to ampicillin while only 5% resistance was seen towards meropenem. Among gram-positive bacteria, 90% of *S. aureus* were resistant to penicillin while all were susceptible to vancomycin and linezolid.

Table 2.Organisms isolated from different clinical samples.

Organism	Pleural fluid	Ascitic fluid	Synovial fluid	Pericardial fluid	Total
<i>Escherichia coli</i>	34	12	4	-	50
<i>Klebsiella pneumoniae</i>	14	3	-	-	17
<i>Citrobacter freundii</i>	5	3	-	-	8
<i>Enterobacter aerogenes</i>	13	4	-	-	17
<i>Pseudomonas aeruginosa</i>	10	4	-	-	14
ACB complex	25	5	1	-	31
<i>Staphylococcus aureus</i>	24	7	7	2	40
<i>Enterococcus faecalis</i>	10	4	1	-	15
<i>Streptococcus pneumoniae</i>	2	-	2	-	4
Total	137	42	15	2	196

Note: ACB complex: *Acinetobacter calcoaceticus-baumannii* complex

Table 3. Antimicrobial resistance pattern of the isolates (%).

Organism	Amikacin	Ampicillin	Ceftriaxone	Cefoxitin	Ofloxacin	Gentamicin	Co-trimoxazole	PIT	Meropenem	Penicillin	Vancomycin	Linezolid
<i>Escherichia coli</i>	20	80	50	-	60	30	50	20	5	-	-	-
<i>Klebsiella pneumoniae</i>	40	-	70	-	65	45	-	30	10	-	-	-
<i>Citrobacter freundii</i>	10	60	65	-	70	75	-	20	20	-	-	-
<i>Enterobacter aerogenes</i>	15	70	65	-	65	72	-	25	20	-	-	-
<i>Pseudomonas aeruginosa</i>	25	-	80	-	80	55	-	40	20	-	-	-
ACB complex	40	-	75	-	60	50	-	40	20	-	-	-
<i>Staphylococcus aureus</i>	20	-	50	30	55	30	70	-	-	90	0	0
<i>Enterococcus faecalis</i>	-	-	-	-	60	35	-	-	-	70	0	0
<i>Streptococcus pneumoniae</i>	0	-	0	-	0	0	0	-	-	0	0	0

Note: PIT: Piperacillin-Tazobactam, ACB complex: *Acinetobacter calcoaceticus-baumannii* complex.

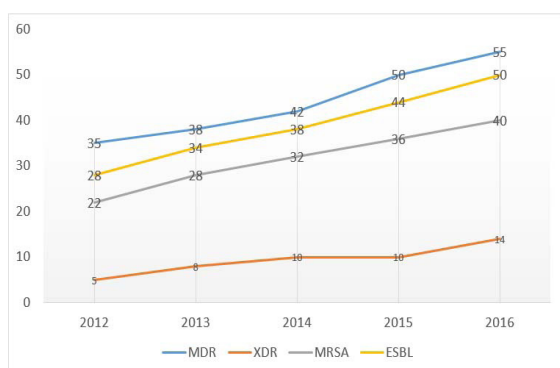


Figure 1. Trends of MDR, XDR, MRSA and ESBL over the years (Percentage).

Note: MDR: multi-drug resistant, XDR: extensively-drug resistant, MRSA: methicillin-resistant *Staphylococcus aureus*, ESBL: extended spectrum  $\beta$ -lactamase

Out of 196 isolates, thirty percent of organisms were MDR, 10% were XDR, 35% of *E. coli* and *K. pneumoniae* were ESBL producer and 30% of *S. aureus* were MRSA. None of the isolates was PDR. The study showed increasing trends of MDR, MRSA and ESBL over the years (Figure 1).

## DISCUSSION

Infections of the sterile body sites, when occurs is a medical emergency and these infections have high morbidity and mortality. Despite its importance, very few studies have been conducted regarding the prevalence and antimicrobial susceptibility pattern of these infections. We analyzed the laboratory records of 1835 body fluid samples submitted for culture and sensitivity that yielded 10.68% positive growth (n=196).

A study conducted by Sujatha R et al.<sup>2</sup> in Kanpur, India showed that 31% of body fluids yielded growth. Similar type of growth rate was obtained by Sharma et al (30%),<sup>5</sup> and Deb et al (21%).<sup>9</sup> The difference might be attributed to the long study period and large sample size in our study.

Among total samples, 64% (n=1172) were pleural fluid, 28% (n=517) ascitic fluid, 7% (n=125) synovial fluid and 1% (n=21) were pericardial fluid. Among 196 positive growth, *E. coli* (n=50, 25%) was the most common organism isolated followed by *S. aureus* (n=40, 20%) and ACB complex (n=31, 16%). Similar results were obtained by Sharma R et al<sup>5</sup> and Sheikhbahei et al.<sup>10</sup> A study conducted by Sujatha R et al. in India also isolated *E. coli* as the most common organism causing infection of body fluids. In contrast to the finding of our study, Deb A et al. isolated *Pseudomonas aeruginosa* as the most common organism causing body fluid infection followed by *A. anitratus*.<sup>9</sup> *E. coli* is the most common bacteria causing body fluid infections.<sup>11</sup>

Antimicrobial susceptibility testing showed variable degree of resistance among organisms. Eighty percent of *E. coli* were resistant to ampicillin, 60% to ofloxacin and 50% to ceftriaxone. However, only 5% of *E. coli* were resistant to meropenem. Among Gram-positive bacteria, all *S. aureus* were susceptible to vancomycin and linezolid, while 90% of them were resistant to penicillin and 70% to co-trimoxazole. *S. pneumoniae* were susceptible to all the antimicrobial agents tested. Similar pattern of antimicrobial susceptibility was reported in various studies (Sujatha et al.<sup>2</sup>, Sharma

et al.<sup>5</sup>). This high resistance level may be due to the inappropriate use of commonly prescribed antibiotics.<sup>12</sup>

Fourty percentage of organisms were MDR, 10% were XDR, 37% of *E. coli* and *K. pneumoniae* were ESBL producer and 30% of *S. aureus* were MRSA. A study conducted by Basak S et al.<sup>13</sup> observed MDR in 37% isolates, XDR in 13% of the isolates, 31% MRSA which is quite similar to the finding of our study. They did not isolate any PDR organisms, which also agrees to our finding. However, they detected ESBL production in 18% of the Gram-negative bacilli, which is lesser than our finding (37%). A study conducted by Shrestha A et al.<sup>14</sup> in Chitwan, Nepal observed 79% MDR and 36% ESBL producing GNB. Our study also showed the increasing trend of MDR, XDR, ESBL and MRSA over the years. Similar results have been reported by studies done worldwide.<sup>4,15</sup> There has been a worldwide increase in emergence of drug resistant organisms in recent years.<sup>15</sup>

MDR bacteria has been well recognized as one of the most important public health problems in current scenario. Treatment outcomes in patients infected with MDR bacteria tend to be worse as compared to those infected with susceptible organisms.<sup>3</sup> MRSA is probably the best example of a prevalent and important MDR bacterium that has successfully transitioned from an almost exclusively nosocomial setting to being widespread in the community. Several researches have concluded that MRSA is increasing as a cause of community-acquired infections.<sup>16-18</sup> Worldwide, the prevalence of MRSA range from 30% to 90% depending upon the type of infections.<sup>3,19</sup> A study conducted at the National Public health laboratory (NPHL), Kathmandu, Nepal reported that 31.57% of *E. coli* were confirmed as Extended Spectrum  $\beta$ -lactamase producers.<sup>20</sup> The Prevalence of ESBL producer worldwide range from 12 to 80%. The epidemiology of ESBL-producing bacteria is becoming more complex with increasingly blurred boundaries between hospitals and the community.<sup>21</sup>

## CONCLUSIONS

The study showed infection rate of 10% among normally sterile body fluids. *E. coli*, *S. aureus* and *ACB complex* are the common organisms. There is an increasing trend of antimicrobial resistance. Routine surveillance of MRSA, ESBL, and multi-drug resistant organisms is essential in proper management of body fluid infections.

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