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**ORIGINAL ARTICLE**DOI: <https://doi.org/10.3329/mediscope.v7i1.47135>**Bacteriological spectrum of urinary, wound and respiratory infection with antibiogram of gram negative bacteria in a Bangladeshi tertiary care hospital****NMW Rahman<sup>1</sup>, B Tanzem<sup>2</sup>, G Nabi<sup>3</sup>, A Mahbub<sup>4</sup>, AMM Al-Amin<sup>5</sup>****Abstract**

Infection remains the main cause of morbidity and mortality in man, particularly in developing areas where it is associated with poverty and overcrowding. Infectious disease cause nearly 25% of all human deaths. This rate of mortality is increasing day by day due to antibiotic resistance, which is a major concern nowadays. Wound, respiratory tract and urinary tract are commonly associated with bacterial infection in both hospital and community settings. Area-specific monitoring studies aimed to gain knowledge about the type of bacterial pathogens responsible for these kinds of infections and resistance pattern of the causative agents may help clinicians to choose correct treatment regimen. So, the present study was aimed to investigate the pattern of bacteria which are responsible for Urinary tract, respiratory tract and wound infection. In addition, we also determined the antibiotic susceptibility profile of gram negative bacteria isolated from the patients who were attending both in and out patient departments at Sir Salimullah Medical College & Mitford Hospital (SSMC & MH) during January, 2009 to December, 2009. In this cross-sectional study, out of 308 clinical samples, a total of 159 (51.62%) samples were found to be positive for bacterial culture. Among the isolates 139 (87.42%) were Gram negative bacteria (*Esch. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Acinetobacter* spp.) and 20 (12.57%) were Gram positive bacteria (*Staphylococcus aureus*, Coagulase negative *Staphylococcus*). Antibiotic susceptibility of gram negative bacteria showed members of the Enterobacteriaceae were 100% sensitive to imipenem while they were found variably resistance to other commonly used antibiotics. We conclude that infections in the wound, respiratory and urinary tract are caused by both gram negative and gram positive bacteria. However, the frequency of gram negative bacteria is higher than the gram positive bacteria for these infections. Gram negative bacteria showed sensitive to imipenem and most of them were resistant to commonly used antibiotics. Therefore, clinicians should choose imipenem for patients who would be unresponsive to commonly used antibiotics.

**Key words:** Antibiotic susceptibility, gram negative and Gram positive bacteria, Urinary, wound and respiratory infection, Bangladesh.

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## Introduction

Bacterial Infection remains the main cause of morbidity and mortality in humans, particularly in developing countries like Bangladesh. This rate of mortality is increasing day by day due to antibiotic resistance, which is a major concern now a day. The term antimicrobial resistance (AMR) refers to the microorganism, that is able to survive in spite of antibiotic treatment. Antimicrobial resistance developed from several intrinsic factors such as point mutation, gene amplification and extrinsic factors like horizontal transfer of resistant gene between bacteria by plasmids, sex pillis or transposons. These Resistant bacterial pathogens are now challenging to the clinicians and researchers which cannot be reduced once developed even by restricting the antibiotic usage. World Health Organization (WHO) recommends for the emergence of hospital and community acquired resistant bacterial infections due to inappropriate and irrational uses of antibiotics in humans and animals for the therapeutic and non-therapeutic purposes (as growth promoters). In addition, some social factors include demographic change; poverty; poor hygienic practices and overcrowding have been referred as the emergence of multidrug resistant (MDR).<sup>1</sup> The multi-drug resistant organisms create serious medical problem globally that has significantly affected the treatment of infectious diseases.<sup>2-5</sup>

The surveillance of previous studies showed bacterial antimicrobial resistance pattern can differ significantly from one country to another even different regions within the same country.<sup>6-9</sup> In Bangladesh, development of multi-drug resistance in clinical isolates of gram negative bacterial species like *Salmonella typhi*, *Pseudomonas* spp. and *Klebsiella* spp. has been also reported.<sup>10-12</sup> The investigation of antibiotic susceptibility is important particularly in developing countries like Bangladesh that do not control antibiotic usage and maintain adequate epidemiological

surveillance. In addition, poverty and overcrowding allows rapid transmission of infectious diseases with resistant organisms in Bangladesh. Thus, we aimed to analyze the causative bacterial agents for wound, respiratory and urinary infection and antibiotic susceptibility of gram negative bacterial pathogen in a tertiary care hospital in Bangladesh. Therefore, the outcome of our study might be important to identify the resistant drugs, which will help the physicians to choose right antibiotics against wound, respiratory and urinary tract infection.

## Materials and methods

This cross-sectional study was carried out in the department of Microbiology, SSMC & MH for a period of one year from January, 2009 to December, 2009. Total 308 samples of wound swab, throat swab and urine were collected from in-patient and out-patient department of Sir Salimullah Medical College & Mitford Hospital. Samples from patients clinically suspected to have urinary tract infections, wound infection and respiratory tract infection were collected. Samples were collected aseptically in sterilized bottles or disposable sterile tubes and submitted to clinical Microbiology laboratory.

## Culture

All wound swabs, urine samples and throat swabs were inoculated in Blood agar and MacConkey agar media. Throat swabs were also inoculated on Chocolate agar media by calibrated loop technique. All the plates were incubated at 37° C aerobically. After overnight incubation, plates checked for presence of suspected pathogens.

## Isolation and identification of organisms

All the organisms were identified by their colony morphology, staining character, pigment production, motility and other relevant biochemical tests as per standard methods.<sup>13,14</sup> Prior to the above mentioned

tests for detection of urinary pathogens from plate, colony count was done by calibrated loop method.<sup>15</sup>

### Media for antibiotic sensitivity test

Mueller-Hinton agar media was used for antimicrobial susceptibility testing for all the bacteria.<sup>16</sup>

### Antimicrobial sensitivity test

All bacterial isolates were tested for antimicrobial susceptibility by disc diffusion method against different antimicrobial agents.<sup>16</sup>

### Interpretation of zone size

Zone of inhibition produced by each was considered into three susceptibility categories namely Sensitive (S), Intermediate (I) and Resistant (R).<sup>17</sup>

### Results

Total 308 samples were collected from patients with suspected wound infection (swab from surgical wound and other wounds), respiratory tract infection (throat swab) and urinary tract infection (urine) from SSMC & MH of which 207 were urine samples, 96 were wound swab samples and 5 were throat swab samples (Table 01). Among the 308 clinical samples, a total of 159 (51.62%) yielded positive result for bacterial culture. Out of 159 isolates, 139 (87.42%) were Gram negative bacteria and 20 (12.57%) were Gram positive bacteria. Among the isolates majority were Esch. coli 115 (72.32%), followed by Coagulase negative Staphylococcus 16 (10.06%), Pseudomonas spp. 10 (6.28%), Klebsiella spp. 8 (5.03%), Proteus spp. 5 (3.14%), Staphylococcus aureus 4 (2.51%) and Acinetobacter spp.1 (0.628%) (Table 02).

**Table 01: Frequency of bacteria isolated from wound, respiratory and urinary infections (n=308).**

Types of sample	Number of tested samples	Number of isolated bacteria
Urine	207	86 (41.54)
Wound swab	96	71 (73.95)
Throat swab	5	2 (40)
Total	308	159 (51.62)

**Note: Figures in parentheses represent percentage.**

In case of urine sample, detected Gram negative bacteria were 85.73% and Gram positive bacteria were 15.11%. Among these bacteria, highest number of isolates were E. coli (74.41%), followed by Coagulase negative Staph (11.62%), Pseudomonas (5.81%), Staphylococcus aureus (3.48%), Klebsiella spp. (2.31%), Proteus (1.16%) and Acinetobacter (1.16%). In case of wound swab Gram negative bacteria was 91.54% and Gram

positive bacteria was 8.45%. Among these maximum number of isolates were E. coli (71.83%), followed by Klebsiella spp. (7.04%), Pseudomonas (7.04%), Proteus (5.63%), Coagulase negative Staph (7.04%) and Staphylococcus aureus (1.40%). Out of 5 throat swab sample only 2 reveals Klebsiella spp. and Coagulase negative Staph (Table 02).

**Table 02: Distribution of isolated bacteria in wound, respiratory and urine samples collected from SSMC & MH (n=159)**

Isolated bacteria	Urine n=86	Wound swab n=71	Throat swab n=2	Total N=159
<i>Esch. coli</i>	64 (74.41)	51 (71.83)	0 (0.00)	115 (72.32)
<i>Klebsiella spp.</i>	2 (2.32)	5 (7.04)	1 (50.00)	8 (5.03)
<i>Proteus spp.</i>	1 (1.16)	4 (5.63)	0 (0.00)	5 (3.14)
<i>Pseudomonas spp.</i>	5 (5.81)	5 (7.04)	0 (0.00)	10 (6.28)
<i>Acinetobacter spp.</i>	1 (1.16)	0 (0.00)	0 (0.00)	1 (0.628)
<b>Total Gram (-ve) Bacteria</b>	<b>73 (85.73)</b>	<b>65 (91.54)</b>	<b>1 (50)</b>	<b>139 (87.42)</b>
<i>Staph. aureus</i>	3 (3.48)	1 (1.40)	0 (0.00)	4 (2.51)
<i>Coagulase neg Staph</i>	10 (11.62)	5 (7.04)	1 (50.00)	16 (10.06)
<b>Total Gram (+ve) Bacteria</b>	<b>13 (15.11)</b>	<b>6 (8.45)</b>	<b>1 (50)</b>	<b>20 (12.57)</b>

Note: Figures in parentheses represent percentage.

In this study antibiogram was only determined only against Gram negative bacteria, which demonstrated that- *E. coli*, *Klebsiella*, *Proteus* and *Pseudomonas* showed around or more than 80% resistance to Amoxicillin, Cephadrine and tetracycline. Moreover, these bacteria were around or more than 60% resistance to Cotrimoxazole, Azithromycin, Aztreonam, Ciprofloxacin and Nalidixic acid. In addition, resistance to Ceftriaxone, Ceftazidime, Cefotaxime, Mecillinam, Gentamicin and Chloramphenicol were around or more than 40%

among these isolated bacteria. We also determined the antibiotic sensitivity pattern of these bacteria. *E. coli* and *Klebsiella spp.* have shown good sensitivity pattern against Netilmicin, Nitrofurantoin and Amikacin, such as, *E. coli* (76%, 81% and 80%) and *Klebsiella spp.* (67%, 34% and 67%); whereas, *Proteus* and *Pseudomonas* showed less sensitivity to these antibiotics. As only one *Acinetobacter* was isolated that's why it has not so significant value regarding antimicrobial sensitivity pattern. All isolates were 100% susceptible to imipenem (Table 03).

**Table 03: Frequency of antimicrobial drug resistance among the Gram negative bacteria isolated from SSMC & MH**

Antimicrobial drug	<i>Esch. coli</i> N=70	<i>Klebsiella</i> <i>spp.</i> N=3	<i>Proteus</i> <i>spp.</i> N=5	<i>Pseudomonas</i> <i>spp.</i> N=10	<i>Acinetobacter</i> <i>spp.</i> N=1
Amoxicillin	55 (78.57)	3 (100)	4 (80)	-	1 (100)
Cephradine	58 (82.85)	3 (100)	4 (80)	-	1 (100)
Ceftriaxone	31 (44.28)	2 (66.66)	3 (60)	6 (60)	1 (100)
Ceftazidime	26 (37.14)	3 (100)	2 (40)	5 (50)	0 (0)
Cefotaxime	29 (41.42)	2 (66.66)	3 (60)	7 (70)	1 (100)
Aztreonam	43 (61.42)	2 (66.66)	3 (60)	7 (70)	1 (100)
Nalidixic Acid	48 (68.57)	2 (66.66)	2 (40)	10 (100)	1 (100)
Ciprofloxacin	42 (60)	2 (66.66)	4 (80)	9 (90)	0 (0)
Tetracycline	51 (72.85)	2 (66.66)	4 (80)	-	-
Cotrimoxazole	45 (64.28)	2 (66.66)	2 (40)	8 (80)	1 (100)
Mecillinam	28 (40)	2 (66.66)	2 (40)	-	-
Azithromycin	39 (55.71)	1 (33.33)	3 (60)	4 (40)	1 (100)
Gentamicin	34 (48.57)	2 (66.66)	1 (20)	8 (80)	1 (100)
Chloramphenicol	27 (38.57)	1 (33.33)	2 (40)	8 (80)	0 (0)
Netilmicin	17 (24.28)	1 (33.33)	3 (60)	6 (60)	1 (100)
Nitrofurantoin	13 (18.57)	2 (66.66)	3 (60)	10 (100)	0 (0)
Amikacin	14 (20)	1 (33.33)	2 (40)	4 (40)	0 (0)
Imipenem	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Note: Figures in parentheses represent percentage  
(-) = Not used.

### Discussion

Out of 308 clinical samples, a total of 159 (51.62%) bacteria were isolated from wound, respiratory and urine samples: 139 (87.42%) Gram negative bacteria and 20 (12.57%) were Gram positive bacteria. Among the isolates, majority were *Esch. coli* 115 (72.32%), followed by Coagulase negative *Staphylococcus* 16 (10.06%), *Pseudomonas* spp. 10 (6.28%), *Klebsiella* spp. 8 (5.03%), *Proteus* spp. 5 (3.14%), *Staphylococcus aureus* 4 (2.51%) and *Acinetobacter* spp. 1 (0.628%). Previous Bangladeshi studies

the evidence of more similar findings with our study. A former Bangladeshi study by Shahidullah et al. (2014) showed that most common isolated bacteria from blood, urine, pus, pericardial fluid, swab from wound, conjunctiva and throat were *Escherichia coli* (40.1%) followed by *Pseudomonas* spp. (30.4%), coagulase negative *Staphylococcus* (19.0%), coagulase positive *Staphylococcus* (5.9%) and beta-haemolytic *Streptococcus* (4.2%).<sup>18</sup> Accordingly, another study by Rahman et al. (2007) also showed majority of the isolated bacteria from Urine, Pus, Sputum,

Vaginal swab, throat swab and Conjunctival swab were *Escherichia coli* (33.33%) followed by *Klebsiella* spp. (27.48%), *Staphylococcus aureus* (17.05%), *Acinetobacter* spp. (8.14%) and *Pseudomonas* spp. (7.12%).<sup>19</sup> Whereas, Chowdhury et al. found the predominant isolated bacteria from pus, wound swab, urine, blood and throat swab were *Staphylococcus aureus* (38.66%), followed by *Escherichia coli* (38%), *Pseudomonas* spp. (13.33%), *Proteus* (8.335), coagulase negative *Staphylococcus* (7.66%), *Serratia* spp. (2.85%), *Klebsiella* spp. (2.00%) and *Acinetobacter* spp. (0.97%).<sup>20</sup>

Among the urine samples, 85.73% was Gram negative bacteria and 15.11% was Gram positive bacteria. From them, *E. coli* (74.41%) was highest isolated bacteria followed by coagulase negative Staph (11.62%), *Pseudomonas* (5.81%), *Staphylococcus aureus* (3.48%), *Klebsiella* spp. (2.31%), *Proteus* (1.16%), *Acinetobacter* (1.16%). Earlier Bangladeshi studies were consistent with our findings. Shahidullah et al. (2014) found most common isolated bacteria in urine were *Escherichia coli* (71.1%) followed by coagulase negative Staph. (15%) and *Pseudomonas* (13.3%).<sup>18</sup> Accordingly, Islam et al. (2012) also found most common isolated organism from urine were 58.3% *Esch. coli* followed by 13.46% *Klebsiella* spp., 12.56% *Proteus* spp. and 11.66% *Pseudomonas* spp.<sup>21</sup> In case of wound swab of present study, we found Gram negative bacteria was 91.54% and Gram positive bacteria was 8.45%. Among these bacteria *E. coli* (71.83%) was highest isolated bacteria followed by *Klebsiella* spp. (7.04%), *Pseudomonas* (7.04%), *Proteus* (5.63%), coagulase negative staph (7.04%) and *Staphylococcus aureus* (1.40%). Similarly, Chowdhury et al.(2013) also found mostly isolated organisms from wound swab were *E. coli* (59.22%), staph. aureus (39.80%), pseudomonas (25.24%), coagulase negative staph (15.53%), proteus (12.62%), klebsiella (1.94%) and acenatobacter (0.97%).<sup>20</sup> How

ever, Shahidullah et al. (2014) had shown that *Pseudomonas* spp. (37.3%) was mostly isolated from pus followed by *E. coli* (31.3%), coagulase negative staph (19.3%) and *Staphylococcus aureus* (7.2%).<sup>18</sup> In addition, among our 5 throat swab samples, only 2 reveals *Klebsiella* spp. and Coagulase negative *Staphylococcus*. Chowdhury et al. (2013) isolated only one staph aureus from throat sample.<sup>20</sup> As the number of samples was less so the result is not so significant.

According to resistance profile of Gram negative bacteria, we found *E. coli*, *Klebsiella*, *Proteus* and *Pseudomonas* shows around or more than 80% resistance to Amoxicillin, Cephadrine and Tetracycline. Besides, resistance to Cotrimoxazole, Azithromycin, Aztreonam, Ciprofloxacin and Nalidixic acid was around or more than 60%. In addition, these bacteria showed around or more than 40% resistance to Ceftriaxone, Ceftazidime, Cefotaxime, Mecillinam, Gentamicin and Chloramphenicol. Previous study by Shahidullah et al. (2014) showed that *Pseudomonas* species was resistant to penicillin, amoxicillin and vancomycin and 50% resistant to cotrimoxazole, cefuroxim, ceftriaxone, piperacillin, azythromycin, cephalixin, netilmycin and ofloxacin.<sup>18</sup> We also determined the antibiotic sensitivity of these bacteria. *E. coli* and *Klebsiella* spp. have shown good sensitivity pattern against Netilmicin, Nitrofurantoin and Amikacin; for *E.coli* (76%, 81% and 80%) and for *Klebsiella* spp (67%, 34% and 67%). While, *Proteus* and *Pseudomonas* showed less sensitivity to these antibiotics. All gram negative bacteria were 100% susceptible to imipenem. This high level of sensitivity to imipenem could be due to its restricted and limited use in the clinical practice. Similar effectiveness of imipenem has also been reported from other countries.<sup>22-24</sup> Chowdhury et al. (2013) also found all the gram negative bacteria were 98-100% imipenem sensitive.<sup>20</sup> The author also found both gram negative and gram positive bacteria showed

high resistance against amoxicillin, ciprofloxacin, co-trimoxazole and ceftriaxone but good susceptibility to gentamicin and levofloxacin.<sup>20</sup> Furthermore, Rahman et al. (2007) also found sensitivity to imipenem was 94-100% for Enterobacteriaceae, 93.0% for Pseudomonas and 97.0% for Acinetobacter spp.<sup>19</sup> This study also reported Enterobacteriaceae was sensitive to third-Generation Cephalosporins like Ceftriaxone, Ceftazidime and Cefotaxime (45-66%), Gentamicin (52.8-67.9%) and Ciprofloxacin (33-40%) but resistant to Ampicillin.<sup>19</sup> This author also found that Acinetobacter was sensitive to third-generation Cephalosporins (50-56%), Ciprofloxacin (40.6%) and to Chloramphenicol, Co-trimoxazole, Cephalexin and Ampicillin (9.3%-34.3%).<sup>19</sup> As only one Acinetobacter was isolated by our study, that's why it did not have a significant value regarding antimicrobial sensitivity pattern. Drug resistance surveillance before the induction of therapy is necessary to guide the appropriate and judicious antibiotic use.

### Conclusion

In conclusion, both gram negative and gram positive bacteria were responsible for wound, respiratory and urinary tract infection, but frequency of infection with Gram negative bacteria was much higher than Gram positive bacteria. All the gram negative bacteria were sensitive to imipenem and most of them showed resistant to commonly used antibiotics. Therefore, clinicians can consider imipenem for patients those who remain unresponsive to commonly used antibiotics.

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