

ORIGINAL ARTICLE

# Resistant Gram-negative bacilli (Red Plague) from ICU in Sohag University Hospital

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## ABSTRACT

**Key words:**

**Multidrug resistant gram-negative bacilli, Red Plague, Tigecycline**

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**Background:** Gram-negative bacteria; such as *Escherichia*, *Klebsiella*, *Enterobacter*, *proteus*, *Pseudomonas* and *Acinetobacter*, are common human pathogens. Now, it is difficult to treat these infections by most of the available antibiotics due to emergence of multidrug resistant Gram-negative bacilli or Red Plague. **Objectives:** This work aims to identify the rate of resistant gram negative bacteria in Sohag University hospital. **Methodology:** The present study was done in Sohag University Hospital, a tertiary care teaching hospital in Upper Egypt, more than 1000 bed capacity. Clinical specimens from the ICU patients were collected aseptically and transferred immediately to the microbiology laboratory for culture and sensitivity during the period from September 2016 to March 2017. Identification and antimicrobial sensitivity of the isolated Gram-negative bacilli were done by Vitek 2 automated system. Susceptibility was confirmed using the disc diffusion (modified Kirby Bauer) method using antimicrobial susceptibility testing discs supplied by (Oxoid, UK). The results were interpreted according to CLSI guidelines. **Results:** Out of 226 isolates from the different clinical specimens; 118 (52.2%) isolates were Gram-negative bacilli. *Escherichia coli* was the most common isolated among these Gram-negative bacilli (53 out of 118 isolates, 44.9%) and was the most common cause of urinary tract infection in ICU, 84% (42 isolates out of 50 urine samples). *Klebsiella pneumoniae* was the commonest cause of pneumonia in ICU (28 out of 34 respiratory isolates, 82.3%). *Pseudomonas spp.*; (17 isolates, 14.4%; *Pseudomonas aeruginosa* 15 isolates and *Pseudomonas stutzeri* 2 isolates) also isolated. *Acinetobacter baumannii* complex (6 isolates, 5.1%), *Serratia liquefaciens* (2 isolates, 1.7%) and *Stenotrophomonas maltophilia* (2 isolates, 1.7%) were the least common in ICU. Nearly all the MDR Gram-negative bacilli isolated in this study were sensitive to Tigecycline. **Conclusion:** This study highlights high rates of multidrug resistant gram negative bacilli. Almost all the isolates were sensitive to Tigecycline. Therefore; antibiotic stewardship, rigorous adherence to infection control guidelines, prevention of antibiotic misuse and continuous surveillance system should be followed to decrease emergence of new resistant strains.

## INTRODUCTION

Gram-negative bacteria; such as *Escherichia*, *Klebsiella*, *Enterobacter*, *proteus*, *Pseudomonas* and *Acinetobacter*, are common human pathogens. These organisms stain pink or red by Gram stain and cause many infections that were treated successfully by antibiotics. Now, it is difficult to treat these infections by most of the available antibiotics due to emergence of multidrug resistant Gram-negative bacilli or Red Plague. There have been as many plagues as wars in history; yet always plagues and wars take people equally by surprise <sup>1</sup>. We have to learn lessons from previous plagues that killed many unprepared human populations. Bubonic plague, or the Black Death killed more than a third of Europe's populations from 1346 to 1351, and the White Plague (tuberculosis) became

epidemic in Europe throughout the 19<sup>th</sup> century. We can prevent the spread of multidrug resistant gram-negative bacilli; the new Red Plague <sup>2</sup>.

Misuse of antibiotics in both humans and animals led to increased rate of antimicrobial resistance especially in developing countries. More than 50% of *E. coli* in China and more than 70% in India were extended spectrum  $\beta$ -lactamase producing strains. <sup>3</sup>

Patients in intensive care units are usually with reduced host defenses and impaired immune responses, multiple invasive procedures and devices distorting the anatomical integrity of the protective barriers making them of the highest occurrence rates of nosocomial infections<sup>4</sup>. Infections with MDR microorganisms compared to sensitive strains increases the cost of treatment per patient 6000-30000 US dollars <sup>5</sup>.

## METHODOLOGY

### Patients and sampling

The present study was done in Sohag University Hospital, a tertiary care teaching hospital in Upper Egypt, more than 1000 bed capacity. Clinical specimens from the ICU were collected aseptically and transferred immediately to the microbiology laboratory for culture and sensitivity during the period from September 2016 to March 2017. All Samples were collected after 48 hours admission in ICU, inoculated on routine culture media and incubated aerobically at 37°C for 24 hours.

**Respiratory secretions:** Thirty four sputum samples and tracheal aspirates (from intubated patients) were collected from patients with respiratory symptoms.

**Urine samples:** Under complete aseptic precautions; urine samples were collected from 50 patients; 5 midstream samples from non catheterized patients, and 45 samples in sterile syringe from the demarcated site of catheterized patients, transported immediately to the laboratory and inoculated on nutrient agar, incubated at 37 °C for 24 hours.

**Swabs** from surgical site infections of 22 patients were collected from deep incisions after skin antiseptis.

**Blood samples** were collected aseptically and injected immediately into adult blood culture bottles (80 ml bottle from Egyptian Diagnostic Media company), incubated aerobically and subcultures were done every 48 hours.

### Species identification and antimicrobial susceptibility testing by Vitek 2

Identification and antimicrobial sensitivity of the isolated Gram-negative bacilli were done by Vitek 2 automated system. Gram-negative identification and antimicrobial susceptibility testing cards were used to determine the susceptibility of the isolates to different antimicrobial agents. Pure subcultures of the isolates were suspended in sterile saline to achieve a turbidity of 0.5 McFarland standards, as measured by the DensiChek turbidity meter (bioMérieux) and used to inoculate the identification cards containing the biochemical substrates. The Vitek 2 compact instrument automatically filled, sealed, and incubated the individual test cards with the prepared culture suspension. Final profile results were compared to the database, generating identification of the unknown organism. The isolates were either susceptible, intermediate susceptible or resistant to the antimicrobials.

### Antimicrobial susceptibility testing by disc diffusion method

Susceptibility was confirmed using the disc diffusion (modified Kirby Bauer) method using antimicrobial susceptibility testing discs supplied by (Oxoid, UK) for the following antibiotics; Amikacin (AK 30 µg), Amoxycillin/clavulanic acid (AMC 30µg), Aztreonam (ATM 30µg), Cefazolin (CZ 30µg), Cefoxitin (Fox 30µg), Cefepime (FEP 30µg), Ceftazidime (CAZ 30µg), Ceftriaxone (CRO 30µg), Ciprofloxacin (CIP 5µg), Gentamicin (CN 120µg), Kanamycin (K 30µg), Levofloxacin (LEV 5µg), Nitrofurantoin (F 300µg), Trimethoprim/Sulphamethoxazole (SXT 25µg), Tigecycline (TGC 15 µg), Imipenem (IPM 10µg), Piperacillin/Tazobactam (TZP 110 µg). The results were interpreted according to CLSI guidelines (6).

## RESULTS

Out of 226 isolates from the different clinical specimens isolated between September 2016 and March 2017, 118 (52.21%) isolates were Gram-negative bacilli isolated from 118 patients in the ICU; 71 male patients aged 18-75 years old and 47 female patients aged 20-71 years old. The isolated bacteria were as follows; *Escherichia coli* (53 isolates, 44.9%), *Klebsiella pneumoniae* (38 isolates, 32.2%), *Pseudomonas spp.* (17 isolates, 14.4%; *Pseudomonas aeruginosa* 15 isolates and *Pseudomonas stutzeri* 2 isolates), *Acinetobacter baumannii complex* (6 isolates, 5.1%), *Serratia liquefaciens* (2 isolates, 1.7%) and *Stenotrophomonas maltophilia* (2 isolates, 1.7%).

The most common isolated pathogen from urine samples was *E. coli* (84%; 42 isolates out of 50 urine samples), while *K. pneumoniae* was the most common isolated from respiratory samples (82.3%; 28 isolates out of 34 respiratory samples). *Pseudomonas aeruginosa* was the most common cause of surgical site infection among Gram negative isolates constituting 59% (13 isolates out of 22 isolates). Surgical site infections usually acquired during surgery not in the ICU. Urinary tract infection was the most common one in ICU in this study (Table 1).

While all *E. coli* and *K. pneumoniae* strains isolated in this study were sensitive to Tigecycline (Table 2), most of them were resistant to penicillins and cephalosporins. Amikacin was effective against 92.4% of *E. coli* isolates (49 out of 53 *E. coli* isolates). All *Serratia* and *Stenotrophomonas spp.* were resistant Tigecycline, (Table 3).

**Table 1: Number and percentages of organisms isolated from different clinical samples**

Sample Organism	Respiratory (n=34, 28.8%)	Urinary (n=50, 42.3 %)	Surgical (n=22, 18.7%)	Blood (n=12, 10.2 %)	Total	P value
<i>E. coli</i>	0	42	6	5	53	0.000
<i>K. pneumoniae</i>	28	5	1	4	38	
<i>P. aeruginosa</i>	2	0	13	0	15	
<i>P. stutzeri</i>	1	1	0	0	2	
<i>A. baumannii</i>	1	1	2	2	6	
<i>S. liquefaciens</i>	0	1	0	1	2	
<i>S. maltophilia</i>	2	0	0	0	2	

*E. coli* and *K. pneumoniae* isolates were statistically significant increased in urinary and respiratory samples respectively, P value= 0.000

**Table 2: Drug resistance pattern of Escherichia, Klebsiella and Pseudomonas species**

Antibiotic/Isolate	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>P. stutzeri</i>
Amikacin	49 S	18 S, 2 I	4 S, 4 I	2 S
Ampicillin	Pan R	Pan R	Pan R	2 S
Ampicillin/Sulbactam	2 S	Pan R	Pan R	2 S
Cefazolin	4 S	Pan R	Pan R	2 R
Cefoxitin	14 S	4 S	Pan R	2 R
Amoxicillin/clavulanic acid	2 S	Pan R	Pan R	2 R
Aztreonam	Pan R	Pan R	Pan R	2 R
Cefepime	6 S	Pan R	2 S	2 S
Ceftazidime	4 S	Pan R	Pan R	2 S
Ceftriaxone	4 S	Pan R	2 S	2 S
Ciprofloxacin	14 S	4 S	6 S	2 S
Gentamicin	26 S	4 S	4 S, 2 I	2 S
Kanamycin	28 S	4 S	2 S	2 S
Levofloxacin	10 S	Pan R	2 S	2 S
Nitrofurantoin	38 S	6 I	Pan R	2 R
Trimethoprim/Sulphamethoxazole	12 S	2 S	2 S	2 S
Tigecycline	All S	All S	6 R	2 S
Imipenem	All S	4 S	4 S	2 S
Piperacillin/Tazobactam	12 S	4 S	Pan R	2 S

S= Sensitive, R= Resistant, I= Intermediate

**Table 3: Drug resistance pattern of Acinetobacter, Serratia and Stenotrophomonas species**

Antibiotic/ Isolate	<i>A. baumannii</i>	<i>S. liquefaciens</i>	<i>S. maltophilia</i>
Amikacin	Pan R	2 S	2 R
Ampicillin	Pan R	2 R	2 R
Ampicillin/Sulbactam	Pan R	2 R	2 R
Cefazolin	Pan R	2 R	2 R
Cefoxitin	Pan R	2 R	2 R
Amoxicillin/clavulanic acid	Pan R	2 R	2 R
Aztreonam	Pan R	2 R	2 R
Cefepime	Pan R	2 R	2 R
Ceftazidime	Pan R	2 R	2 R
Ceftriaxone	Pan R	2 R	2 R
Ciprofloxacin	Pan R	2 R	2 R
Gentamicin	3 S, 3 R	2 S	2 R
Kanamycin	Pan R	2 R	2 R
Levofloxacin	Pan R	2 R	2 S
Nitrofurantoin	Pan R	2 I	2 R
Trimethoprim/Sulphamethoxazole	Pan R	2 R	2 S
Tigecycline	3 I, 3 R	2 R	2 R
Imipenem	Pan R	2 R	2 R
Piperacillin/Tazobactam	Pan R	2 R	2 R

S= Sensitive, R= Resistant, I= Intermediate

## DISCUSSION

During the last years, a global increase in MDR Gram-negative bacteria was observed with limited supply of new effective antimicrobial agents. They are very common in ICU settings with high rate of mortality. They are an important source for septicemia, pneumonia and urinary tract infections. Reporting of such isolates increased in last few years due to misuse of antibiotics<sup>7</sup>.

In high income countries; mortality rate in ICUs varies from 8% to 18%. Rates could be higher up to 60% in low income countries<sup>8</sup>. Hence, this study was carried out to assess this problem in our hospital.

In the present study; the most common Gram negative species isolated was *E. coli* (44.9%). This result was like that of Sameera et al.<sup>9</sup>. All *E. coli* isolates were sensitive to Imepenem, Tigecycline and 49 isolates 92.5% were sensitive to amikacin. Almost all isolates were resistant to  $\beta$ - lactam antibiotics. A study in 2009 was done by Hawser et al.<sup>3</sup> revealed that > 50% of *E. coli* in China and > 70% in India were extended spectrum B-lactamase producing strains.

The second common isolate was *K. pneumoniae* (32.2%), all of them were sensitive to Tigecycline. All isolates were resistant to B- lactam antibiotics and Levofloxacin. *K. pneumoniae* is naturally resistant to aminopenicillins<sup>8</sup>.

Also, the results of this study were similar to that observed by Kumarasamy et al.<sup>10</sup> who recorded that there was a high prevalence of resistance to common antibiotics and carbapenems in more than 50% of *Klebsilla pneumoniae* isolates and more than 20% of *E. coli* isolates.

*Pseudomonas aeruginosa* has a strong tendency to become multidrug resistant pathogen. It was not uncommon in this study and about 35% of the isolates were resistant to penicillins, cephalosporines and aztreonam. It was acquired during surgery because swabs were taken 48 hours after admission to ICU.

The two strains of *Pseudomonas stutzeri* were sensitive to about 75% of the used antibiotics. *Pseudomonas stutzeri* is Gram negative bacterium rarely cause infections; but isolated as an opportunistic pathogen in immunocompromised patients<sup>11</sup>.

Resistant *Acinetobacter* spp. was increased in ICUs; six (5.1%) isolates were isolated in this study, three of them (50%) were resistant to Tigecycline. Pan resistant *Acinetobacter baumannii* infections are common among critically ill patients in intensive care units worldwide<sup>12</sup>. Nearly all of them were multidrug resistant, this is in accordance with Bayram and Balci<sup>13</sup>.

*Acinetobacter* isolates constitute only 5.1% in this study in contrast to the results of Patwardhan et al.<sup>14</sup>

who recorded that *Acinetobacter* was a major cause of ICU infections in his study.

Two strains of *Stenotrophomonas maltophilia* were isolated in this study and were sensitive only to Levofloxacin and Trimethoprim/ Sulphamethoxazole.

Frequent isolation of gram negative organisms may be due to their wide prevalence in the hospital environment. In addition, their frequent resistance to antibiotics may play a role in their persistence and spread.

Nearly all the isolates in this study were sensitive to Tigecycline. Tigecycline is a bacteriostatic agent that inhibits protein synthesis by binding to the 30S ribosomal subunit of bacteria and frequently included in a combination regimen. Evidence has demonstrated its efficacy for the treatment of MDR Gram-negative infections.<sup>15</sup>

## CONCLUSION

Multidrug resistant organisms (MDROs) are resistant to at least one of the available classes of antibiotics. MDROs are increasing in many populations all over the world, so the prevention and control of these pathogens should be a national priority.

The mainstay to control the spread of antibiotic-resistant pathogens in the ICU is rigorous adherence to infection control guidelines and prevention of antibiotic misuse. Strictly following antibiotic policies result in reduced drug costs and due to continuous changes in antibacterial susceptibility patterns, periodical antibacterial sensitivity assessment in ICUs should be mandatory. Emphasis was laid on various infection control measures such as adequate hand washing techniques, aseptic measures for all procedures, antibiotic cycling and health education for the health care personnel.

Identification of the susceptibility patterns of the isolated organisms is very essential to select empirical therapy and improve patient outcome.

Nearly Tigecycline is highly effective against all isolates in this study. It should be used judiciously and reserved for cases with resistant pathogens for other antibiotics.

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