RESEARCH ARTICLE

GRAM-NEGATIVE BACTERIA IN SOME CLINICAL SPECIMENS AND THEIR ANTIBIOTICS RESISTANCE PROFILES, SANA’A, YEMEN


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Abstract

Gram-negative bacteria (GNB) have emerged globally as significant public health problems owing to their high resistance to antibiotics, particularly in developing countries such as Yemen. Therefore, this study aimed to determine the antibiotic resistance profile of GNB isolated from clinical samples in Sana’a City, Yemen. A total of 420 clinical samples, including vaginal swabs, urine, and pus, were collected from patients who attended some hospitals in Sana’a City, Yemen, in 2018. The collected specimens were examined according to standard microbiological methods, and the isolated bacteria were tested against some antibacterial agents using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Among 420 specimens, the prevalence of gram-negative bacteria infections was 49.76%. A high rate of GNB was reported among the age group of 41–50 years old (57.97%), female patients (52.19%), those who came from rural areas (60.0%), and those who were uneducated (86.56%). The vaginal specimens exhibited a higher prevalence of bacterial bacteria (60.42%), followed by urine (51.28%) and pus (35.0%). Escherichia coli accounted for the greatest proportion of bacteria recovered from the study’s patients (36.62%), followed by Klebsiella pneumoniae (18.66%), Enterobacter sp. (12.32%), Acinetobacter sp. (10.92%), Pseudomonas aeruginosa (9.15%), Citrobacter sp. (6.34%), and Proteus mirabilis (5.99%). E. coli was highly resistant to imipenem and enrofloxacin. Similarly, K. pneumoniae and P. aeruginosa were highly resistant to imipenem and norfloxacin. About 62.86% of Enterobacter sp. isolates were resistant to imipenem. In addition, 83.33–78.97% of Acinetobacter sp. isolates were resistant to imipenem and meropenem. Citrobacter sp. had high resistance to norfloxacin. Additionally, over 50% of P. mirabilis isolates were resistant to ofloxacin, norfloxacin, cefuroxime, and ceftriaxone. The conclusion is that there exists a significant proportion of GNB prevalence and antibacterial resistance among the study subjects. Therefore, multisectoral approach is needed for implementing effective strategies to reduce the transmission of antibiotic-resistant bacteria among the community.

Keywords: Antibiotic resistant, Gram-negative bacteria, Pus, Urine, Vaginal Swabs, Sana’a, Yemen.

Introduction

The emergence and rapid spread of antimicrobial-resistant pathogenic bacteria is a global health challenge [1]. The primary factors contributing to the heightened incidence of antibiotic-resistant microorganisms are unnecessarily high levels of antibiotic use, excessive utilization of broad-spectrum antibiotics, irrational drug use, substandard drug quality, inadequate sanitation,
malnutrition, inadequate healthcare systems, and inadequate control over antibiotic use and stewardship programs [2-4].

Gram-negative bacteria can cause infections in healthcare settings, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Gram-negative infections include those caused by Klebsiella sp., Acinetobacter sp., Pseudomonas aeruginosa, and Escherichia coli, as well as many other less common bacteria that affect millions of people worldwide and are related to a lack of sanitation [5-6].

Gram-negative bacteria are resistant to multiple drugs and are increasingly resistant to the most available antibiotics. These bacteria produce extended-spectrum beta-lactamase (ESBL) enzymes that are capable of hydrolyzing penicillin, broad-spectrum cephalosporin, and monobactams [7]. Accordingly, ESBL enzyme-producing bacteria can resist penicillin, broad-spectrum cephalosporins, and monobactams [8].

Several reports documented the rate of gram-negative bacteria resistance to antimicrobial agents. A recent report recorded that 13% of E. coli and Klebsiella sp., 17% of P. aeruginosa, and 74% of A. baumannii in intensive-care units were multidrug-resistant globally [9]. In a similar, it was reported that the rate of resistance to ciprofloxacin varied from 8.4% to 92.9% for E. coli and from 4.1% to 79.4% for K. pneumoniae in countries reporting to the Global Antimicrobial Resistance and Use Surveillance System. Also, E. coli resistance to third-generation cephalosporins was recorded at 36.0% in 49 countries [10].

In Yemen, with poor resources, most physicians attempt to decrease the cost of laboratory examinations by treating patients using empirical therapy according to previous clinical experience without the use of antibiotic testing. These factors contribute to an increase in mortality and morbidity due to treatment failures and a lack of effective therapy [11-13].

The prevalence of antimicrobial-resistant bacteria in Yemen was documented by investigators [14-17]. It was found that P. aeruginosa and E. coli isolated from wound infection had a high resistance to tetracycline, erythromycin, nalidixic acid, and nitrofurantoin [4].

Recently, a study by observed that the gram-negative bacteria recorded a high resistance to ampicillin, nalidixic acid, ceftriaxone, and cotrimoxazole [15]. However, not enough information exists about the resistance of gram-negative bacteria to some common antibiotics in Sana’a city, Yemen. Therefore, the goal of the present study was to assess the antibiotic resistant profile of isolated gram-negative bacteria from some clinical samples in Sana’a City-Yemen.

Materials and Methods

Study design and period

This cross-sectional study was carried out at Al-Sabeen, Al-Thowra, and Al-Gomhori hospitals situated in the Sana’a capital of Yemen from February to June 2018.

Data collection

A structured questionnaire was used to collect the required data from participating individuals. The needed data, such as age, gender, patients’ resident area, specimen source or type, and site of infection, were gathered via face-to-face interviews and recorded on a questionnaire sheet.

Inclusion and exclusion criteria

The inclusion criteria were represented by patients who didn’t use antibiotics before the week, while patients who used antibiotics for the past seven days were excluded.

Specimen Collection

Specimens were collected from patients aged between 19 and 75 years old by using sterile equipment and an aseptic technique. The clinical samples, including vaginal swabs, urine, semen, and pus, were collected from 70 patients diagnosed with infection based on clinical signs and laboratory investigations. Specimens were taken from patients according to microbiological standard procedures by medical nurses and laboratory technicians and immediately transported to the laboratory for microbiological examination [18].

Microbiological examination

Clinical samples were cultured in an appropriate medium according to standard methods for bacteria isolation and identification of negative bacteria. The positive cultures were performed using conventional biochemical tests, including the oxidase test, catalase test, kligler’s iron agar (KIA), sulfide indole motility (SIM), citrate agar test, and carbohydrate fermentation [18].

Antimicrobial susceptibility test

All the isolated bacteria were tested against different antimicrobial agents by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, and the interpretations were carried out according to the Clinical and Laboratory Standards Institute guidelines [19]. The antibiotic discs that were used include Enrofloxacin (EX, 10 μg), Norfloxacin (NX, 10 μg), Ofloxacin (OF, 5 μg), Tetracycline (TE, 10 μg), Cefuroxime (CXM, 30 μg), Chloramphenicol (C, 30 μg), Imipenem (IMP, 10 μg), Cefizoxime (CZX, 30 μg), and Meropenem (MRP, 10 μg). The inhibition zones were measured using a ruler and interpreted by company instructions [19].
Ethics statement

This study was approved by the Ethics Research Committee of Queen Arwa University and permitted by the subject hospital’s administration in Sana’a City. Informed consent was obtained from all the participants.

Results

**Demographic and clinical characteristics**

A total of 420 participants enrolled in this study. The high rate of specimens was collected from patients in the age group of 41–50 years (32.85%), females (54.29%), who came from urban areas (58.33%), hold a secondary school certificate (30.48%), and urine (30%), as illustrated in Table 1.

| Table (1): Demographic and clinical characteristics of participated patients |
|------------------|-----------------|-----------------|-----------------|
| Variables        | Frequency (%)   | Rate (%)        |
| Age (in years)   |                 |                 |
| 19-30            | 78              | 18.57           |
| 31-40            | 102             | 24.29           |
| 41-50            | 138             | 32.85           |
| 51-75            | 102             | 24.29           |
| Gender           |                 |                 |
| Male             | 192             | 45.71           |
| Female           | 228             | 54.29           |
| Residence area   |                 |                 |
| Rural            | 175             | 41.67           |
| Urban            | 245             | 58.33           |
| Educational level|                 |                 |
| Illiterate       | 67              | 15.95           |
| Primary          | 116             | 27.62           |
| Secondary        | 128             | 30.48           |
| Graduate         | 109             | 25.95           |
| Urine            | 156             | 37.14           |
| Pus              | 120             | 28.57           |
| Vaginal swab     | 144             | 34.29           |
| Type of specimen  |                 |                 |
| Urine            | 156             | 37.14           |
| Pus              | 120             | 28.57           |
| Vaginal swab     | 144             | 34.29           |
| Non-GNB growth No. (%) | 45 (57.69) |
| Non-GNB growth No. (%) | 52 (54.74) |

The overall rate of gram-negative bacteria in this study was reported in 209 (49.76%) specimens, while 211 (50.24%) specimens showed negative growth (Figure 1).

Table 2 shows a high rate of pathogenic bacteria was observed among patients in the age group of 41–50 years old (57.97%), female patients (52.19%), who live in rural areas (60.0%), and the uneducated (86.56%). As per the specimen types, it can be observed that the vaginal specimens exhibited a higher prevalence of pathogenic bacteria (60.42%), followed by urine specimens (51.28%), and pus (35.0%).

![Fig. 1: Prevalence rate of gram-negative bacteria in study specimens](https://ejua.net)

![Fig. 2: Proportion rate of gram-negative bacteria prevalent](https://ejua.net)
Table 3 shows the antimicrobial susceptibility of gram-negative bacteria isolated from clinical specimens. *E. coli* showed higher resistance to imipenem (97.22%), followed by tetracycline (84.62%), and enrofloxacin (78.85%). Additionally, it was moderately resistant to chloramphenicol and meropenem (55.77 %).

However, the *K. pneumoniae* isolates were more sensitive to ofloxacin (71.69%). In contrast, it was completely resistant to imipenem (100%) and highly resistant to norfloxacin (92.46%). Cefuroxime was moderately effective against *P. aeruginosa* (53.85%). In contrast, 88.46% of the *P. aeruginosa* isolates were not affected by norfloxacin or imipenem. In addition, most *P. aeruginosa* isolates were moderately resistant to the last antibiotic used (Table 3).

Furthermore, 62.86% of the *Enterobacter* sp. isolates showed resistance to chloramphenicol and imipenem. *Acinetobacter* sp. showed resistance to imipenem, meropenem, and tetracycline at rates of 83.33%, 80.65%, and 78.97%, respectively. *Citrobacter* sp. showed a high resistance to chloramphenicol (77.82%), tetracycline (66.73%), and norfloxacin (61.11%). Additionally, more than half of the *P. mirabilis* isolates were resistant to ofloxacin (64.71%), norfloxacin (64.71%), tetracycline and cefuroxime (58.82% each), and cefotaxime (52.94%) (Table 3).

### Discussion
The study comprised 420 participants. Of these, a significant proportion (32.85) of specimens were obtained from patients aged between 41 and 50 years; 54.29% of them were female; 56.33% of them were from urban regions; 30.48% had a secondary school certificate; and 30% of the specimens originated from urine. This is similar to the results of a recent investigation [20, 21].

In this study, the overall rate of gram-negative bacteria was 49.76%. This finding is lower than reports at 77.1% in Ecuador [22] and 79.4%. In addition, these data are higher than those reported in Ethiopia (16.9%) [20], Mexico (19.1%) [23], and Nepal (17%) [24].

The high rate of GNB prevalence in our study may be due to several reasons, including misuse and overuse of antimicrobials, lack of access to clean water, poor practical hygiene, poor infection and disease prevention and control, poor access to quality, affordable medicines, and lack of awareness and knowledge.

Regarding age, a high rate of GNB was 57.97% observed in the current study among the age group of 41–50 years. This study is supported by earlier studies that documented that patients over 45 years of age had a high rate of microbial infections [24, 26]. Older people may get sick more often because their bodies do not work well enough, and their immune systems become weaker [27].

The present data showed that GNB were more significant among female patients than among male patients. However, as this study found, most studies indicated that UTIs in women predominate over UTIs in men, with UTIs being identified as diseases in women [25]. Poor personal cleanliness and anatomical predispositions that allow bacteria to enter the bladder may be the main causes, compared to men. In different reports, gram-negative infections were more frequently observed in males [27, 28].

### Table (3): Antibacterial susceptibility profiles for isolated gram-negative bacteria

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>C (%)</th>
<th>OF (%)</th>
<th>TE (%)</th>
<th>EX (%)</th>
<th>NX (%)</th>
<th>CXM (%)</th>
<th>IMP (%)</th>
<th>CZX (%)</th>
<th>MRP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> (104)</td>
<td>46 (44.23)</td>
<td>24 (23.07)</td>
<td>16 (15.38)</td>
<td>22 (21.15)</td>
<td>24 (23.07)</td>
<td>10 (26.32)</td>
<td>3 (2.88)</td>
<td>25 (24.04)</td>
<td>46 (44.23)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (53)</td>
<td>58 (55.77)</td>
<td>80 (76.93)</td>
<td>88 (84.62)</td>
<td>82 (78.85)</td>
<td>80 (76.93)</td>
<td>28 (73.68)</td>
<td>107 (97.22)</td>
<td>89 (75.96)</td>
<td>58 (55.77)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> (26)</td>
<td>27 (50.95)</td>
<td>38 (71.69)</td>
<td>19 (35.85)</td>
<td>20 (37.73)</td>
<td>4 (7.54)</td>
<td>15 (28.30)</td>
<td>0 (0)</td>
<td>16 (30.19)</td>
<td>19 (35.85)</td>
</tr>
<tr>
<td>Enterobacter sp. (35)</td>
<td>26 (49.05)</td>
<td>15 (28.31)</td>
<td>34 (64.15)</td>
<td>33 (62.27)</td>
<td>49 (92.46)</td>
<td>38 (71.70)</td>
<td>53 (100)</td>
<td>37 (69.81)</td>
<td>34 (64.15)</td>
</tr>
<tr>
<td>Acinetobacter sp. (31)</td>
<td>11 (42.31)</td>
<td>7 (26.92)</td>
<td>8 (30.77)</td>
<td>7 (26.92)</td>
<td>3 (11.54)</td>
<td>14 (53.85)</td>
<td>3 (11.54)</td>
<td>10 (38.46)</td>
<td>12 (46.15)</td>
</tr>
<tr>
<td>Citrobacter sp. (18)</td>
<td>13 (37.14)</td>
<td>31 (88.57)</td>
<td>16 (45.78)</td>
<td>25 (71.43)</td>
<td>26 (74.28)</td>
<td>20 (57.14)</td>
<td>13 (37.14)</td>
<td>28 (80.00)</td>
<td>27 (77.14)</td>
</tr>
<tr>
<td><em>P. mirabilis</em> (17)</td>
<td>22 (62.86)</td>
<td>4 (11.43)</td>
<td>19 (54.28)</td>
<td>10 (28.57)</td>
<td>11 (25.72)</td>
<td>15 (42.84)</td>
<td>22 (62.86)</td>
<td>7 (20.00)</td>
<td>8 (22.86)</td>
</tr>
</tbody>
</table>

C= Chloramphenicol; OF= Ofloxacin; TE= Tetracycline; EX= Enrofloxacin; NX= Norfloxacin; CXM= Cefuroxime; IMP= Imipenem; CZX= Cefotaxime; MRP= Meropenem. S= Sensitive, R= Resistance, NA= Not applicable.

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**Table 3**: Antibacterial susceptibility profiles for isolated gram-negative bacteria.
In the current study, uneducated patients were more exposed to GNB infection than others. Several studies have been conducted in different Yemeni regions that support this finding [29-30]. Education enhances the likelihood of a long and healthy life and has the potential to reduce health disparities [31].

According to specimen type, the frequency of GNB was recorded in 51.28% of urine specimens. A comparable study conducted in Cairo, Egypt found that the most common source of microbial illnesses was urine specimens [25]. In addition, several studies conducted in Yemen supported this data [32-33]. Previous studies have indicated that gram-negative bacteria are responsible for approximately 90% of UTI cases, whereas gram-positive bacteria account for only 10% of cases [34]. Approximately 150 million people worldwide are afflicted with urinary tract infections (UTIs), which are among the most widespread microbiological illnesses associated with hospitals and communities [35].

The prevalence of GNB in the vaginal specimens was 60.42%. This finding is higher than the prevalence of GNB reported in a similar study [36]. Variations in the prevalence rates of bacterial vaginosis have been linked to sociodemographic characteristics, sexual behavior, reproductive health information, and behavioral and genital hygiene. Additionally, an increase in the rate of vaginal infections has been reported in Yemen [37-39].

Most bacteria recovered from the patients in this study were E. coli (36.62%), followed by K. pneumoniae (18.66%), Enterobacter sp. (12.32%), and Acinetobacter spp. (10.92%). P. aeruginosa (9.15%), Citrobacter sp. (6.34%), and P. mirabilis (5.99%), respectively. This is in agreement with previous results obtained from different countries [20, 26, 40] which found that E. coli, K. pneumoniae, and Proteus sp. were the most frequently isolated gram-negative bacteria. Another study revealed that K. pneumoniae and P. aeruginosa are the most frequently isolated GNB infections [28].

The observable variations in the overall prevalence and occurrence of gram-negative bacteria may be attributed to the sources and numbers of clinical samples collected, the nature of infections, patient types or wards where the samples were obtained, and geographical disparities employed in each study.

E. coli showed greater resistance to imipenem (97.22%), tetracycline (84.62%), and enrofloxacin (78.85%). Moreover, resistance to meropenem and chloramphenicol was only moderate at 55.77% for each drug. These results are consistent with those of earlier studies that reported resistance rates to these antibiotics ranging from 70% to 90%. Furthermore, it was found that over 50% of isolated E. coli strains were resistant to 10 of the 18 tested antibiotics [34]. In contrast, at rates of 92.3% and 89.4%, E. coli was extremely susceptible to imipenem and meropenem, respectively. Additionally, E. coli showed different levels of resistance to cefuroxime (87.95%) [23], tetracycline (76.7%) [36], and chloramphenicol (46%) [24].

In this study, resistance of K. pneumoniae isolates to imipenem (100%) and norfloxacin (92.46%) was observed. A similar study reported that 59.7% and 44.7% of Klebsiella spp. isolates were resistant to meropenem and imipenem, respectively, [34]. In addition, resistance rates of over 65% of K. pneumoniae isolates have been reported for cefoperazone, ceftriaxone, cefotaxime, ciprofloxacin, norfloxacin, and ofloxacin [24]. Additionally, it was observed that between 64.3% and 81.55% of isolated K. pneumoniae were resistant to the cefuroxime antibiotic [23, 41]. Moreover, K. pneumoniae has been found to be highly resistant to tetracycline [42].

These data demonstrated that the highest resistance rate of P. aeruginosa isolates was observed for norfloxacin and imipenem, corresponding to 88.46% in each case, and the last administered antibiotics showed moderate resistance. The resistance of P. aeruginosa to imipenem has been observed in more than 60% of similar studies [23, 34]. Additionally, P. aeruginosa showed resistance rates of 50%, 63%, and 75% to meropenem, norfloxacin, and ofloxacin, respectively [24].

Furthermore, the highest resistance rate of the Enterobacter sp. isolates was 62.86% for both chloramphenicol and imipenem. This result is in agreement with the results of a previous study [24]. Moreover, a significant proportion of Acinetobacter sp. (83.33%, 80.65%, and 78.97%, respectively) showed resistance to imipenem, meropenem, and tetracycline. A similar report showed high rates of resistance to almost all tested antibiotics in Acinetobacter spp. [34].

Citrobacter sp. showed high resistance rates towards chloramphenicol (77.82%), tetracycline (66.73%), and norfloxacin (61.11%). This result is in agreement with previous findings reported in some countries [20, 43, 44].

This finding revealed that 64.71% of the P. mirabilis isolates were resistant to ofloxacin and norfloxacin, 58.82% to tetracycline and cefuroxime, and 52.94% to cefizoxime. This result aligns with the recent finding that the resistance rate of P. mirabilis was 67% for both ofloxacin and norfloxacin [25]. Additionally, 82.35% of the P. mirabilis isolates from this study exhibited sensitivity to meropenem and imipenem. These results are in agreement with previous reports showing that Proteus sp. had a sensitivity rate of 60.2% and 53.3% for meropenem and imipenem, respectively [34]. Currently, meropenem has been found to be the most effective antibiotic against Proteus sp. at a sensitivity rate of 100% [45].
The rationale behind the disparity in susceptibility or resistance of bacterial isolates can be attributed to the extent to which these isolates are subjected to factors that result in the emergence of resistance. These include the indiscriminate administration of antibiotics for extended periods without requiring medical guidance. Furthermore, low health standards, poor drug quality, the fact that physicians have issued unnecessary antibiotic prescriptions without conducting adequate susceptibility testing on bacteria, and the rapid and uncontrolled use of antimicrobials in agriculture and farming have all contributed to the escalation of this issue.

Conclusion

This study found a high prevalence of Gram-negative bacteria, which means that there might be a big problem for the health system in the area studied. Additionally, most of the isolated GNB were resistant to imipenem antibiotics at different levels, except for *Citrobacter* sp. and *P. mirabilis* bacteria. There may be neglected factors contributing to the increasing prevalence of GNB among study patients. Therefore, epidemiological studies can focus on the prevalence, possible associated risk factors, and antimicrobial resistance patterns of GNB in a large-scale sample. Effective policies and procedures are crucial for controlling and preventing the dissemination of antibiotic-resistant bacteria in the community.

Conflict of Interest

The authors declare that this article's content has no conflict of interest.

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References


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قياس المقاومة للبكتيريا سالبة الجرام في بعض العينات السريرية وم nyaوائمةها للمضادات الحيوية، صنعاء، اليمن

الخلاصة

ظهرت البكتيريا سالبة الجرام في بعض العينات السريرية موقعًا ضروريًا لمعالجة المرضى، و لذلك، فإن هذه الدراسة تهدف إلى تحديد مقاومة المضادات الحيوية لدى البكتيريا سالبة الجرام، وتحديد العوامل المحيطة. اجريت هذه الدراسة محلية في مدينة صنعاء، اليمن، من عام 2018 إلى 2020. تم استخدام طرق الميكروبيولوجية التقليدية لإنتاج البكتيريا والتجزئة. وأظهرت النتائج أن البكتيريا مستهلكة للمضادات الحيوية. تشكل الإشريكية القولونية النسبة الأكبر من البكتيريا سالبة الجرام بنسبة 58.79%، و ثم كاليستوبلاستيس، و Citrobacter، و Enterobacter sp. و Pseudomonas aeruginosa. 

كلمة المفتاحية: مقاومة المضادات الحيوية، البكتيريا سالبة الجرام، قيحة، بول، مسحات مختبرية، صنعاء، اليمن

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