

Detection of Potential ESBL, Ampc, and Metallo-Beta-Lactamase Producers (MDR-GNB) Through In-vitro Susceptibility Testing of Genitourinary Specimens in a Community-based Pilot Study

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ABSTRACT

This is a pilot study, which is intended to represent a basis for future studies pertinent to multidrug-resistant antimicrobial gram-negative (MDR-GNB) bacteria that commonly prevail in Iraqi community and hospital settings. In the present study, we scrutinize the antimicrobial phenotypic resistant patterns of commonly isolated Enterobacteriaceae (i.e., *E. coli*, *E. aerogenes*, *K. oxytoca*, *P. mirabilis*) from urine, wound samples, and high vaginal swabs in the Iraqi community. One hundred fifty- six (156) urine, genital lesions, and high vaginal swab samples were obtained from a community population in a cross-sectional study held in Baghdad at a private Lab from December 2020 to the end of April 2021. These specimens were isolated and identified according to conventional methods. Antimicrobial sensitivity tests were done according to the Kirby-Bauer method, and interpretation of results was made with reference to CLSI Standards for Antimicrobial Susceptibility Testing. Gram-negative bacilli were isolated from 80 (about 51%) of genitourinary specimens sent to the laboratory for culture and sensitivity. *E. coli* and *E. aerogenes* showed the most frequent bacterial isolate in genitourinary samples (60% and 31.25%, respectively). Complete resistance (i.e. 100%) to Amoxicillin-Clavulanic acid and Cefotaxime and high resistance (i.e. 97.5%) to Cefixime, Ceftriaxone, and Ceftazidime by these bacterial strains were observed. Assessment of Enterobacteriaceae antibiotic resistance for both of β - lactams and Carbapenems showed that 25% of these strains were having resistance to both groups of antibiotics. As a conclusion; Enterobacteriaceae multi-drug resistance reflects potential ESBL, Ampc, and carbapenemase activity in Iraq.

Keywords: MDR-GNB, genitourinary, antibiotic susceptibility.

1. INTRODUCTION

Multi-drug resistant gram-negative- bacteria (MDR-GNB) prevalence is on the rise worldwide [1] and has left a negative impact on the quality of health-care, which is clear from healthcare- associated infections (HAI) as in surgical site infections (SSI) in various categories of patients and hospitals [2]. This stands in clear contrast to

prior studies where some surgical site infections revealed a poly-microbial picture [3]. Such a situation resulted from resistant mechanisms intrinsic to gram-negative bacteria [1]. It follows that bacterial genetic machinery led to emergence of Beta-lactamase hydrolytic enzyme activity in what is now known as (ESBL), (AmpC), (Carbapenemase) [4,5], the latter was first detected in a Swedish tourist in 2009 New Delhi, India, hence its

designated name (NDM-1) [6-8], however, it should be noted that an *Escherichia coli* strains in patients with no travel history to the Indian subcontinent had been isolated and was suspected to be (NDM-1) when it demonstrated non-susceptibility to Carbapenem and third-generation antimicrobials [9].

The NDM-1 can be conveyed to human intestinal normal flora, further compounding the problem [10], and in connection to these antibiotics prescribed for humans can change the overall natural inhabitants of human intestinal flora [11,12]. The accumulative effects of the aforementioned environmental and health factors along with the emergence of other Carbapenem-resistant gram-negative bacteria such as the non-fermentative Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) [13] in the ICU led to a desperate search for solutions to the challenge of treating (MDR-GNB). The aim of the current study is to explore a preliminary assessment of multi-drug resistant (MDR) Enterobacteriaceae in clinical samples of the genitourinary system in women living in Baghdad, Iraq.

2. MATERIALS AND METHODS

A cross-sectional, community-based study was held in Baghdad / Private clinic from December 2020 to the end of April 2021 were 156 different specimens from genito-urinary system including urine, vaginal lesions, and genital wound swabs from patients suffered from symptoms and signs of different bacterial infections were enrolled for the detection of Enterobacteriaceae in these specimens and assessing their antibiotic resistance pattern.

Midstream-urine clean catch samples (MSU) were obtained in sterile containers from individuals in the community. Wound (from the genitourinary system) and high vaginal swabs were aseptically taken with non-toxic sterile cotton swabs. All specimens were inoculated according to conventional methods [16-20].

Anti-microbial susceptibility tests were run for the bacterial isolates according to the Kirby-Bauer method. Results were interpreted according to clinical and laboratory standards institutes (CLSI) Performance Standards for Antimicrobial Susceptibility Testing [21].

The ethical approval for current study was obtained from the Institutional Review Board (IRB) at Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, IRAQ. Patient's informed consent was obtained from all the patients for using their samples in the current study. The authors declare that they have no conflict of interest associated with this publication.

3. RESULTS

Among 156 specimens sent for culture and sensitivity to the laboratory, gram-negative bacilli were isolated from 80 (about 51%) of them- that were identified later as Enterobacteriaceae "Table (1)".

The frequency of bacterial isolates occurrence in specimens is shown in "Figure (1)", *E. coli* shows the most frequent bacterial isolate in vaginal and urine samples (70% and 66.6%, respectively), while *E. aerogenes* showed the most frequent isolate among vaginal swab samples (56.6%) in the current study.

The proportion of resistance for these bacterial isolates to common β -lactams and Carbapenems used in Iraq is shown in "Table (2)".

Enterobacteriaceae antibiotic resistance for β -lactams and for both of β -lactams and Carbapenems is demonstrated in "Figure (2)".

4. DISCUSSION

The observed results in this pilot study can be dichotomized on the basis of statistical results and the community setting as the source of bacterial samples in relation to documented observations. The phenotypic antimicrobial sensitivity tests reveal a resistant pattern of Enterobacteriaceae which are clear against the third generation cephalosporins with 100 % antibiotic resistance to Amoxicillin-Clavulanic acid and Cefotaxime. Other 3rd generation cephalosporins showed also a high resistance pattern (i.e. 97.5%). These readings can be accounted for on the basis of the hydrolytic enzyme activity of the Enterobacteriaceae microorganisms against extended spectrum cephalosporins to which the above mentioned isolates are non-susceptible. Basically bacterial enzymatic hydrolysis is brought about through extended spectrum-Beta-lactamase (ESBL) activity and (AmpC) production [4,5]. Several bacteria of the Enterobacteriaceae group do possess extended spectrum-Beta-lactamase [22] activity which explains their resistant pattern against the third generation antimicrobials.

Our antibiogram demonstrates about 15% resistance to Imipenem. Moreover, about 22.5% of resistance against another Carbapenem antimicrobial was noticed, namely meropenem. This is due to metallo-beta lactamase (MBL) enzyme production in these resistant strains [23].

The NDM-1 is another plasmid-mediated transmissible carbapenemase gene, which readily spreads among bacterial populations and has gained much

attention recently given the nature of protective hydrolytic activity of Beta-lactamase in this lethal strain that overcomes bactericidal effects of B-lactams. Hence the term metallo-Beta-lactamase applies to (NDM-1) (MDR) strain possessing a lethal nature capable of acquiring additional resistant genes [23] which is evidently clear from the antimicrobial sensitivity test results in current study. The potential presence of (NDM-1) bacterial strain isolates in this study cannot be discounted given that many Iraqi citizens have in recent years travelled to the Indian subcontinent to seek medical consultation and even subjected to surgical procedures [4].

It therefore becomes necessary to run lab based confirmatory tests to pick up (ESBL) producing gram negative strains [22,24,25]. In future Iraqi labs. implementing antimicrobial tests for resistant strains may face limitations in terms of (PCR) genotyping of (MDR-GNB) suspected of having (NDM-1) and other resistant genes. For the time being, and probably in the imminent future, treatment of infections in general will be based on phenotypic antibiograms and bacterial species identification.

5. CONCLUSIONS

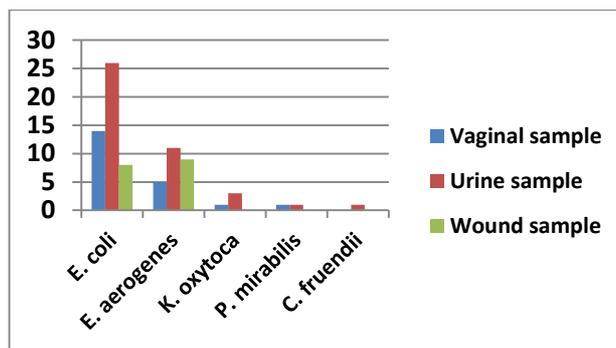
Enterobacteriaceae multi-drug resistant bacteria isolated from genitourinary tract in current study reflects potential ESBL, Ampc, and carbapenemase activity.

6. FIGURES AND TABLES

Table 1. Type of isolated Enterobacteriaceae from genitourinary samples

Sample	No.	Escherichia coli	Enterobacter aerogenes	Klebsiella oxytoca	Proteus mirabilis	Citrobacter freundii
Vaginal	21	14	5	1	1	0
Urine	42	26	11	3	1	1
Wound	17	8	9	0	0	0

Total	80 (100%)	48 (60%)	25 (31.25%)	4 (5%)	2 (2.5%)	1 (1.25%)
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Anti-biotic *	No. of sensitive strains (n=80)	%	No. of resistant strains (n=80)	%	95% Confidence interval
Amx/Clv	0	0%	80	100%	0.0000 to 0.0451
CTX	0	0%	80	100%	0.0000 to 0.0451
CRO	2	2.5%	78	97.5%	0.0030 to 0.0874
CFM	2	2.5%	78	97.5%	0.0030 to 0.0874
CFZ	2	2.5%	78	97.5%	0.0030 to 0.0874
MEM	62	77.5%	18	22.5%	0.6679 to 0.8609
IMI	68	85%	12	15%	0.7526 to 0.9200

*Amx/Clv= Amoxicillin/Clavulanic acid, CTX= Cefotaxime, CRO= Ceftriaxone, CFM= Cefixime, CFZ= Ceftazidime, MEM= Meropenem, IMI= Imipenem

Figure 1. Frequency of bacterial isolates occurrence in genitourinary specimens

Table 2. Resistance of Enterobacteriaceae to common β -lactams and Carbapenems used in Iraq

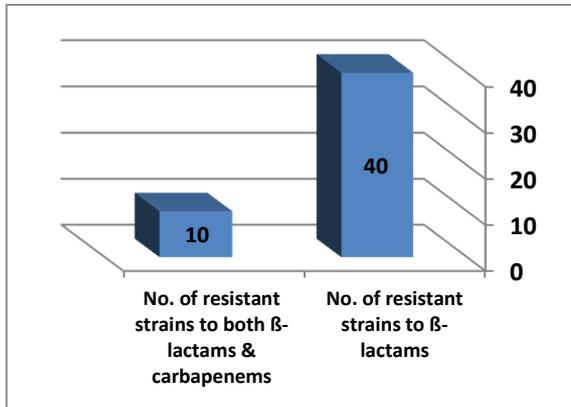


Figure 2. No. of Enterobacteriaceae strain with antibiotic resistance for β -lactams and for both of β -lactams & Carbapenems.

AUTHORS' CONTRIBUTION

Author DA selects the clinical cases and made the primary diagnosis, sent samples to the laboratory, and design the study. Authors AJ and SA made the laboratory diagnosis including isolation and identification of the bacteria, write the initial manuscript, and make the statistical analyses. All authors have read the full manuscript and participate in writing the final manuscript.

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