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RESEARCH ARTICLE
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Occurrence Of CTX-M I gene in Beta lactam resistance Neisseria gonorrhoeae isolated Form women with endocervical infection

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ABSTRACT

Background: Gonococcus is one of the virtually bacteria related to sexually transmitted diseases with high genetic variability, making it more prone to the acquisition of antibacterial resistance particularly to extended-spectrum β-lactamases (ESBLs). In the last years, the most prevalent ESBLs are of the CTX-M type.

Aim: To investigate the occurrence of CTX-M I gene in Beta lactam resistance Endocervical infection-related Neisseria gonorrhoeae isolated from female patients.

Methods: Fifteen Neisseria gonorrhoeae isolates that previously recovered from endocervical swab samples of women with bacterial vaginosis were used in this study. All bacterial isolates and they're resistant to different antibiotics were investigated by VITEK 2 microbial identification system. After plasmid DNA extraction, monoplex-PCR with primer sequences targeting plasmid - mediated - blaCTX-MI resistance gene was used.

Results: Out of Fifteen isolates of resistant Neisseria gonorrhoeae, the plasmid-mediated blaCTX-MI resistant gene was detected in 9 (60%) with amplicon product (499 pb).

Conclusions: The emergence of the CTX-M I gene in Neisseria gonorrhoeae is a major threat to the current beta lactam antibiotics that are used in the treatment of gonorrhea in our country.

Keywords: *Neisseria gonorrhoeae, endocervical infection, Extended-spectrum β-lactamases (ESBLs), blaCTX-MI.*

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INTRODUCTION

Gonococcus, also known as Neisseria gonorrhoeae, is a fastidious Gram-negative diplococcus that is one of the first known human pathogenic bacteria. They are usually observed in pairs, and when viewed under a microscope, their nearby flattened sides give them the appearance of kidneys (1,2).

For growth, it needs a medium that is rich in nutrients, and it prefers a humid climate with a 5% carbon dioxide level. The bacteria can definitely survive at lower temperatures, but their preferred growth range is 35–37°C. Once accessible, it oxidizes glucose and generates oxidase and catalase. (3).

The lower reproductive tract's Bartholin's and Skene glands, urethra, cervix, anal canal, pharynx, and conjunctiva are among the mucosal sites where the pathogen N. Gonorrhea can be acquired (4).

After chlamydial infection, gonorrhea is the second most common STI, and men are more likely to experience its symptoms and seek medical attention for them. On the other hand, gonococcal cervicitis patients rarely display indications of inflammation. (5).

Bacteria that produce extended spectrum beta-lactamase (ESBL) are common causes of nosocomial and community-acquired infections. ESBL enzymes, since its discovery in 1992, Cefotaximases (CTX-M) enzymes encompass a growing fast family of enzymes that appear to have a greater ability to spread and cause outbreaks in several parts of the world; the CTX-M family has evolved into the most common ESBL enzymes in many Gram-negative bacteria. (6)

The name CTX consider the forceful hydrolytic activity of these β-lactamases toward cefotaxime. CTX-M β- lactamases hydrolyze cefotaxime and ceftriaxone better than they do ceftazidime. Five major phylogenetic groups made up of more than 60 blaCTX-M variants have been identified within the CTX-M family. Each group consists of a number of distinct varieties, the most of which are restricted to a small number of geographical regions while the minority is scattered worldwide. (7)

CTX-M-14 and CTX-M-15 were the most frequently isolated variants worldwide; the preponderance of CTX-M-15 across geographical regions and in varied bacterial isolates has fueled the rapid spread of CTX-M-positive ESBL-producing bacteria thus far (8).

Ceftriaxone and ceftriaxone plus azithromycin dual therapy confirmed treatment failures have been recorded more frequently in recent years. This circumstance puts the last first-line medicines currently in use in grave jeopardy and increases the likelihood that gonorrhea may soon be incurable (9).

However, there is no data in our country on the occurrence of the CTX-M I gene produced by Neisseria gonorrhoeae. So, the current objectives of this study were to determine the occurrence of blaCTX-MI -resistance genes in Neisseria gonorrhoeae isolated from females.

MATERIALS AND METHODS

Bacterial strain

A retrospective study was undertaken on archived 15 Neisseria gonorrhoeae isolates which were previously recovered from endocervical swab samples of women with bacterial vaginosis. All bacterial isolates were estimated to have Ceftriaxone, Cefotaxime and Ceftazidime as well as Aztreonam and Imipenem resistance by VITEK 2 microbial identification system according to the procedure suggested by the manufacturing company (bio-Merieux). These bacteria were diagnosed and classified in the Medical Microbiology department at the faculty of medicine, AL -Nahrain university.

Plasmid DNA extraction Protocol

Neisseria gonorrhoeae was harvested by using Luria-Bertani broth media, after centrifugation (8,000) rpm for two minutes ; the pellet was collected and the supernatant was thrown away. Plasmid extraction of the Neisseria gonorrhoeae was performed as described by company instruction (Wizard® Plus Minipreps DNA Purification System, Promega)

Primers that target the plasmid-mediated blaCTX-MI resistance gene were used in a monoplex PCR (Table I). The 35-cycle amplification profile consisted of 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min. The thermocycling conditions were as follows: initial denaturation at 94°C for 7 min., followed by a 35-cycle amplification profile.

Final elongation was taking place for 10 minutes at 72°C. PCR products were run on a 1% (wt/vol) agarose gel for 1.5 hours at a voltage of 7 V/cm (Merck, Germany). Concurrently, a molecular marker (1-kb DNA ladder; Bioneer) was run. After the gel was stained with 1 g/ml ethidium bromide, bands were seen and captured on camera under UV light (Promega, USA).

TABLE 1: Primer sequences for molecular detection of plasmid-mediated blaCTX-MI -resistance gene.

blaCTX-MI	Sequences	Products	Reference
F	GACGATGTCACTGGCTGAGC	499 pb	(10)
R	AGC CGC CGACGCTAATAC A		

RESULTS

Fifteen isolates of Neisseria gonorrhoeae that showed resistance to Ceftriaxone, Cefotaxime and Ceftazidime as well as Aztreonam and Imipenem resistance were screened for the presence of the plasmid-mediated blaCTX-MI resistant gene by PCR. There was 9 blaCTX-MI (60%) among 15 isolates of Neisseria gonorrhoeae positive for this gene with amplicon product (499bp). Figure (1)

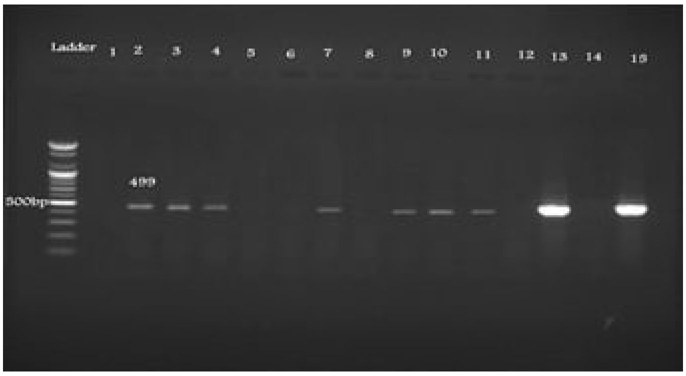

FIGURE 1: Gel electrophoresis of PCR positive products. Lane MW:100bp DNA ladder; lane NC: Negative control (DW); lane (2-4,7,9-11 ,13,15): Neisseria gonorrhoeae positive isolates for blaCTX-MI (499bp); lane (1% agarose, 7 v/cm2,1.5hrs).

DISCUSSIONS

The most widespread infection in women induced by N. gonorrhoeae is cervicitis, but it has the potential to spread and infect the pelvis. Cervicitis typically has no symptoms. If symptoms do exist, they can range from mucopurulent discharge to pruritus and are generally non-specific. The cervix may be normal-appearing or discharge-prone (11).

Since the development of CTX-M ESBLs has similar clinical effects to that of other ESBLs, carbapenems are the only effective -lactam antibiotics for the treatment of severe infections brought on by producer pathogens (12). High rates of co-resistance to potentially active medicines, such as fluoroquinolones and aminoglycosides, have also been documented for enterobacterial strains with CTX-M enzymes, which is similar to what was shown with enterobacterial strains producing TEM and SHV type ESBLs. (13).

More substantially and discouragingly, Neisseria gonorrhoeae is among the most likely bacteria to evolve into incurable superbug's bacteria from developing resistance to every known antibiotic, posing a public health threat that has gained much attention. In humans, cases of developed resistance against beta-lactam antibiotics and others are increasingly documented in Neisseria gonorrhoeae (14).

The Centers for Disease Control and Prevention (CDC) classifies N. gonorrhoeae as an “urgent threat” due to the emergence of antimicrobial resistance and multidrug resistance (15).

Indeed, many reports in the last years of XDR resistant Neisseria gonorrhoeae raised alarm around the globe and ushered in the start of an era of pan-resistant Neisseria gonorrhoeae (16).

Data that deal with the sexually transmitted diseases showed that misused and self-medication, particularly in STDs lead to continuous exposure to therapy, with continuous exposure to antibiotics, which apply selection on Neisseria gonorrhoeae, this augmented by the bacterial ability to acquire resistance genes from other bacteria (via horizontal gene transfer), thus admission the emergence of strain variants that carry diverse resistance traits to many antibiotics especially beta-lactam antibiotics (17).

In addition to tetracycline, many studies have shown that the existence of CTX-M type ESBLs is frequently related with co-resistance phenotypes, particularly to fluoroquinolones and aminoglycosides, as well as trimethoprim/sulfamethoxazole co-resistance, which is frequently seen among TEM and SHV type ESBLs (18).

The emergence of co-resistance between CTX-M- producing isolates with fluoroquinolones and aminoglycosides raises serious concerns and urges experts to issue a warning about improper public usage of ESBLs, notably in Gonococcal infection (19).

This study demonstrates a remarkably high level of CTX-M I gene in Neisseria gonorrhoeae isolated from cervicitis, it has become increasingly resistant to practically all kinds of antibiotics and is considered a severe issue due to its changing multi-drug resistant profile in Neisseria gonorrhoeae, Ultimately, such finding will help in the design of specific intervention strategies to guide empiric choice of antimicrobials for gonococcal infection.

Our study provides the framework for future studies to assess the molecular surveillance based on a larger sample size to contribute the plasmid-mediated in the transfer and the occurrence of CTX-M type ESBLs resistance in Neisseria gonorrhoeae.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors (Thanaa R, Abdulrahman, Ahmed S, Hassain and Jabbar S. hassan) made a substantial contribution to the conception, design, or the analysis and interpretation of the data; and to drafting the article or revising it critically for important intellectual content. Thanaa R, Abdulrahman and Ahmed S, Hassain confirm the authenticity of all the raw data. All authors have read and approved the final manuscript

Ethics approval and consent to participate

Due to the use of bacterial isolates in manuscript

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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