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Original Article

Emergence of Neisseria gonorrhoeae Isolates with Decreased Susceptibility to Cefixime and Ceftriaxone from the Eastern Cape Province, South Africa

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ABSTRACT

Background: In South Africa, third generation cephalosporins (cefriaxone and cefixime) remain the treatment of choice for patients with gonorrhoea. The study sought to ascertain the susceptibility patterns of *N. gonorrhoeae* isolated from patients in Mthatha, Eastern Cape. **Methods:** A total of 103 stored isolates were obtained during a cross-sectional study conducted between 2010 and 2012 at the Mbekweni Health Centre and the Nelson Mandela Academic Complex. The identification of *N. gonorrhoeae* isolates was confirmed using carbohydrate utilization tests. β-lactamase tests were performed using the chromogenic cephalosporin test (Nitrocefin, Oxoid). Minimum Inhibitory Concentrations (MICs) to spectinomycin, ceftriaxone, cefixime, kanamycin and penicillin were determined using the agar dilution method following the Clinical and Laboratory Standards Institute (CLSI) guidelines and interpreted using the recommendations of the World Health Organization (WHO). *Staphylococcus aureus* ATCC 29,213; *N. gonorrhoeae* ATCC 49,226 and the 2008 WHO *N. gonorrhoeae* reference strains F, G, K, N, O and P were used as controls. **Results:** Of the 103 isolates, 48 (46.6%) were susceptible to kanamycin; 75 (72.8%) were susceptible to spectinomycin whilst 28 (27.2%) had spectinomycin MICs of 64 mg/L; 63 (61.2%) were penicillinase producers and 40 (38.8%) isolates were chromosomally mediated penicillin resistant. All isolates (100%) displayed decreased susceptibility to ceftriaxone (MIC ≥0.125 mg/L) and cefixime (MIC ≥ 0.25 mg/L). **Conclusion:** Decrease susceptibility of *N. gonorrhoeae* to third-generation cephalosporins in Mthatha is a major challenge. Spectinomycin may be considered as an effective option. However, continued and broader surveillance of antimicrobial resistance is warranted in order to amend the current treatment guideline.

Keywords: *Neisseria gonorrhoeae, Antimicrobial resistance, Cephalosporins.*

RÉSUMÉ

Contexte : En Afrique du Sud, les céphalosporines de troisième génération (ceftriaxone et céfixime) restent un traitement de choix pour les patients infectés par la gonorrhée. Cette étude vise à vérifier le profil de sensibilité de *N.gonorrhoeae* isolé chez des patients à Mthatha, Cap-Oriental. **Méthodologie :** Un total de 103 isolats conservés, ont été obtenus lors d'une étude transversale menée entre 2010 et 2012 au centre de santé Mbekweni et à l'hôpital universitaire Nelson Mandela. L'identification des isolats de *N. gonorrehoeae* a été confirmée à l'aide de tests d'utilisation des glucides. Des tests β-lactamase ont été réalisés à l'aide du test de céphalosporines chromogène (nitrocéfine, Oxoid). La concentration minimale inhibitrice (CMI) de la spectinomycine, de la ceftriaxone, de la céfixime, de la kanamycine et de la pénicilline a été déterminée à l'aide d'une dilution sur gélose suivant les lignes directrices du Clinical and Laboratory Standards Institute (CLSI) et interprétée suivant les recommandations de l'Organisation mondiale de la santé (OMS). *Staphylocoque doré* ATCC 29,213; *N. gonorrhoeae* ATCC 49,226 et les souches de références WHO *N. gonorrhoeae* F, G, K, N, O et P ont été utilisées comme cas témoins. **Résultats :** Parmi les 103 isolats, 48 (46.6%) étaient sensibles à la kanamycine, 75(72.8%) étaient sensibles à la spectinomycine tandis que 28 (27.2%) avaient une CMI à la spectinomycine de 64 mg/L; 63(61.2%) étaient des producteurs de pénicillinase et 40(38.8%) isolats présentaient une résistance à médiation chromosomique à la pénicilline. Tous les isolats (100%) ont montré une sensibilité réduite à la ceftriaxone (CIM ≥0.125 mg/L) et à la céfixime (CIM ≥ 0.25 mg/L). **Conclusion :** Une sensibilité réduite de *N.gonorrhoeae* aux céphalosporines de troisième génération représente un défi de taille. La spectinomycine peut être considérée comme une option efficace. Toutefois, une surveillance continue et plus vaste de la résistance antimicrobienne doit être garantie afin de modifier les lignes directrices actuelles du traitement.

Mots clés : *Neisseria gonorrhoeae ,résistance antimicrobienne, céphalosporines.*

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INTRODUCTION

The increase in resistance of *N. gonorrhoeae* to all classes of antimicrobials is a serious concern and requires research for alternative options and combinations for its appropriate management. Current treatment options for gonorrhoea are therefore diminishing. As a response, the surveillance of the antimicrobial resistance (AMR) of *N. gonorrhoeae* helps to establish and maintain the efficacy of standard treatment methods. Surveillance should be continued in order to reveal the emergence of new resistant strains and to monitor the changing patterns of resistance so that treatment recommendations can be updated to assist in disease control.

It was until 2008 when the first-line therapy for *N. gonorrhoeae* was changed in South Africa from ciprofloxacin to third generation extended-spectrum cephalosporins (ESCs) (1, 2). This new policy guideline suggested the use of a single 250 mg intramuscular dose of ceftriaxone or a single-dose oral cefixime to replace fluoroquinolones for the treatment of presumptive gonorrhoea (1, 2).

Unfortunately, the emergence and spread of strains of *N. Gonorrhoeae* with decreased susceptibility or displaying full resistance to ESCs were shortly after reported from the western Pacific region initially and thereafter in Europe (3, 4). The first two verified cases of *N. gonorrhoeae* resistant to oral third-generation ESCs in South Africa were reported by Lewis et al. in 2013 (5). These two strains were obtained from men who have sex with men (MSM).

Studies have shown that the alteration of the penA gene encoding for penicillin-binding protein 2 (PBP 2) was the key mechanism for both decreased susceptibility and resistance to third-generation ESCs. In addition, an increase in the minimum inhibitory concentrations (MIC) of the strains of *N. gonorrhoeae* to third-generation ESCs was associated with mutations in the promoter and/or coding sequence of the mtrR gene with subsequent increase in the expression of the MtrCDE efflux pump (3, 4, 6).

Due to increased prevalent cases of resistance of *N. gonorrhoeae* to oral cefixime globally, CDC recommends combination therapy with ceftriaxone 250 mg intramuscularly and either azithromycin 1 g orally as a single dose or doxycycline 100 mg orally twice daily for 7 days as the most reliably effective treatment for uncomplicated gonorrhoea. CDC no longer recommends cefixime at any dose, as a first-line regimen for treatment of gonococcal infections. If cefixime is used as an alternative agent, then the patient should return in 1 week for a test-of-cure at the site of infection. Since third-generation ESCs have been used to treat patients suspected of having gonorrhoea in the province of the Eastern Cape, surveillance data are lacking in order to support the continuous use of this treatment guideline. Hence, the present study sought to ascertain the susceptibility patterns of *N. gonorrhoeae* isolated from patients in Mthatha, Eastern Cape.

MATERIALS AND METHODS

Patients and strains of N. gonorrhoeae:

A total of 103 stored isolates at -70°C in Tryptic soy broth (Difco laboratories, Detroit, MI) containing 20% glycerol were used. Those clinical isolates were obtained during a cross-sectional study conducted between 2010 and 2012 at the Mbekweni Health Centre (a primary healthcare facility) and the Nelson Mandela Academic Hospital (a tertiary level hospital). Patients were presented either with male urethritis syndrome (MUS) or vaginal discharge syndrome (VDS) at the time of enrolment. Written informed consent was obtained from each patient and the present study was approved by the Ethics Committee of the College of Health Sciences at Walter Sisulu University (Ref: 042/2012).

Confirmatory identification of N. gonorrhoeae:

Stored presumptive isolates of N. *gonorrhoeae* were subcultured in New York City plates [GC agar base supplemented with yeast autolysate, lincomycin, colistin, Amphotericin B, trimethoprim, (Oxoid Ltd., Basingstoke, UK), and lysed horse blood]. Inoculated plates were incubated for 48 hours at 37°C in a CO_2 incubator. Gram-negative, oxidase-positive organisms were identified as being *N. gonorrhoeae* by means of carbohydrate utilization tests.

Anti-microbial Susceptibility Testing:

Beta-lactamase tests were performed using the chromogenic cephalosporin test (Nitrocefin, Oxoid). MICs to penicillin, spectinomycin, ceftriaxone and cifixime were determined using the CLSI methodology (7). MICs for kanamycin were performed using the agar dilution method and interpreted using the World Health Organization (WHO) recommendations (8). *Staphylococcus aureus* ATCC 29,213; *N. gonorrhoeae* ATCC 49,226 and the 2008 WHO *N. gonorrhoeae* reference strains F, G, K, N, O and P were used as controls. All MIC tests were performed in triplicate.

Susceptibility of *N. gonorrhoeae* was defined as follows (9-11):

Penicillin susceptible: MIC ≤0.06 mg/L.

PEN-R: chromosomally mediated penicillin resistant; MIC ≥2 mg/L in the absence of beta-lactamase production.

PPNG: positive for beta-lactamase production.

Kanamycin susceptible: MIC ≤32 mg/L.

Kanamycin intermediate: MIC = 64 mg/L.

Kanamycin resistant: MIC ≥128 mg/L.

Spectinomycin susceptible: MIC ≤32 mg/L.

Spectinomycin resistant: MIC ≥128 mg/L.

Ceftriaxone susceptible: MIC ≤ 0.064 mg/L.

Ceftriaxone decreased susceptibility: MIC ≥0.125 mg/L

Cefixime susceptible: MIC ≤ 0.064 mg/L.

Cefixime decreased susceptibility: MIC ≥ 0.25 mg/L

RESULTS & DISCUSSION

The MICs of *N. gonorrhoeae* isolates to the various antimicrobials tested are shown in Table 1. Of 103 isolates, 15 (14.6%) were intermediate and 40 (38.8%) were resistant to kanamycin.

Seventy-five (72.8%) were susceptible to spectinomycin whilst 28 (27.2%) displayed MIC = 64 mg/mL for spectinomycin.

All isolates were resistant to penicillin with 63 out of 103 (61.2%) being PPNG and 40 (38.8%) were chromosomally mediated penicillin resistant (Pen-R).

All isolates 103 isolates displayed decreased susceptibility to ceftriaxone and cefixime – 30(29.1%) isolates had cefriaxone MIC = 0.125 mg/L whilst the remaining 73(70.9%) isolates had ceftriaxone MIC = 0.25 mg/L; all 103 isolates had cefixime MIC = 0.25 mg/L.

Table 1: Activity of 5 Antimicrobial Agents on *Neisseria gonorrhoeae* Isolates from Mthatha between 2010 and 2012 (n = 103)

Antimicrobial agents	**n(%) of strains of *N. gonorrhoeae* with MIC (mg/L)**					
	0.125	0.25	16	32	64	128
Kanamycin			15(14.6)	33(32)	15 (14.6)	40 (38.8)
Spectinomycin			59 (57.3)	16(15.5)	28(27.2)	
Penicillin	6 (5.8)	9 (8.7)	16 (15.5)	40 (38.8)	12(11.7)	20(19.4)
Ceftriaxone	30 (29.1)	73(70.9)				
Cefixime		103(100)				

The use of kanamycin in the treatment of gonorrhoea has been extensive in some parts of Africa, mainly Zimbabwe and Mozambique (2, 9), both countries sharing borders with South Africa. In South Africa, however, aminoglycosides such as kanamycin and amikacin are used for the management of patients with multidrug-resistant tuberculosis (MDR-TB) but not patients with gonorrhoea. The high rate of kanamycin resistant-*Neisseria gonorrhoeae* observed in this present study is not surprising either in a setting where resistance among isolates of *Mycobacterium tuberculosis* is relatively high in the community. Resistance to amikacin, a commonly used aminoglycoside in South Africa for the treatment of other infectious diseases (i.e. infections caused by Gram negative bacteria) in addition to the treatment of MDR-TB, induces almost complete cross-resistance to kanamycin (12).

The study showed that spectinomycin is an effective treatment option in patients with gonorrhoea. Although 27.2% of isolates displayed MIC = 64 mg/mL for spectinomycin, no single case of resistance was confirmed. The use of spectinomycin in the treatment of patients infected with *N. gonorrhoeae* has been limited in Africa mainly due to the high cost and lack of availability of this antimicrobial agent (2). In addition, another challenge in the use of spectinomycin is the lack of good clinical response and poor efficacy among patients diagnosed with gonococcal pharyngitis (3, 13, 14). In general terms, gonococcal infection of the pharynx is more difficult to

eradicate as compared to infections involving urogenital and anorectal sites (14).

Penicillin should no longer be regarded as an alternative therapeutic agent among patients with gonorrhoea in South Africa; and the present study has confirmed that all isolates were resistant to penicillin. Other historical antimicrobial agents used in the treatment of gonorrhoea in South Africa include sulfonamides, tetracycline, and ciprofloxacin. The antimicrobial activity of these agents, however was not assessed in this present study (2).

All isolates displayed decreased susceptibility to third generation extended-spectrum cephalosporins (ceftriaxone and cefixime). The emergence and spread of strains of *N. Gonorrhoeae* with decreased susceptibility or displaying full resistance to ESCs have been widely reported mainly in the western Pacific region and in Europe (3, 4). In South Africa, two confirmed cases of *N. gonorrhoeae* resistant to oral third-generation ESCs were reported in Gauteng among MSM patients in 2013 (5). Although 3rd generation ESCs remain the treatment of choice for patients with gonorrhoea in South Africa, the US Centers for Diseases Control and Prevention (CDC) no longer recommends cefixime at any dose as a first-line regimen. According to the CDC recommendations and many other authorities, ceftriaxone monotherapy should be avoided; and a combination therapy with ceftriaxone and either azithromycin or doxycycline should become the first line treatment of choice.

CONCLUSION

This is the first report from the province of Eastern Cape in South Africa on the emerging cases of *N. gonorrhoeae* displaying decreased susceptibility to 3rd generation ESCs. Since these isolates were collected between 2010 and 2012, it is possible that the findings from this study could not be reflecting the true picture of the current susceptibility patterns of *N. gonorrhoeae* to 3rd generation ESCs. The study shows the need for continuous and broader surveillance to inform the current treatment guidelines. Another limitation of this study is the fact that clinical response to 3rd generation ESCs was not assessed. We did not also assess the MICs of *N. gonorrhoeae* to Azithromycin, a recommended drug by CDC to be used as part of the combination therapy with ceftriaxone. However, the study has shown that spectinomycin may be considered as an effective treatment option when managing patients with gonorrhoea, with the exception of pharyngeal gonorrhoea.

Competing Interest:

Authors declare that they have no competing interest.

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