Cluster of multidrug-resistant Neisseria gonorrhoeae with reduced susceptibility to the newer cefalosporins in Northern Greece

Eva Tzelepi1*, Maria Daniilidou2, Vivi Miriagou1, Eirini Siatravani1, Efthimia Pavlidou2 and Alexandros Flemmaakis3

1Laboratory of Bacteriology of the Hellenic Pasteur Institute, National Reference Centre for Neisseria gonorrhoeae, 127 Vas. Sofias Ave., 11521 Athens, Greece; 2Laboratory of Microbiology, Venereal and Skin Diseases Hospital of Thessaloniki, Thessaloniki, Greece; 3Microbiology Laboratory of the ‘Andreas Sygros’ Hospital for Skin and Venereal Diseases, Athens, Greece

Keywords: gonorrhoea, therapy, STD

*Corresponding author. Tel: +30-210-64-78-810; Fax: +30-210-64-40-171; E-mail: tzelepi@pasteur.gr

Sir,

Therapeutic options for gonococcal infection are nowadays limited due to the spread of gonococci resistant to a wide variety of antimicrobials. The dramatic increments in the rates of quinolone-resistant gonococci and the current shortage of spectinomycin leave the newer cefalosporins as the last safe choice among the drugs that are currently recommended as first-line treatment. Therefore, increasing reports of reduced in vitro activities of expanded-spectrum cephalosporins against Neisseria gonorrhoeae are of serious concern.1–3

The aim of this report from the Greek National Reference Centre for Neisseria gonorrhoeae (NRCNG) is to be aware of the presence, in Northern Greece, of a cluster of multidrug-resistant gonococci that exhibit decreased susceptibility to the newer cefalosporins.

From December 2006 through January 2008, a total of 195 gonococcal strains were submitted to the NRCNG, and all were characterized by serovar identification with the GC serotyping panel (BACTUS AE, Sweden) and tested for susceptibility to various antimicrobials using the Etest (AB Biodisk, Sweden). By analysing the results, a group of 17 isolates showed cefotaxime MICs 0.25–1 mg/L, as opposed to ≤0.002–0.125 mg/L for the remaining gonococci. By extending susceptibility testing to other cephalosporins, these isolates were found to exhibit raised MICs to ceftriaxone and cefotaxime also, while being highly resistant to cefuroxime and non-susceptible to cefoxitin, cefazidine and cefepime. Cefalotin and aztreonam MIC50 values were increased up to 8-fold when compared with the ATCC 49266 strain, which was tested in parallel. This group of cefalosporin decreased susceptibility (CEDS) isolates consisted of non-penicillinase-producing, fluoroquinolone-resistant and multidrug-resistant strains with low-level resistance to penicillin G, tetracycline, erythromycin and chloramphenicol (Table 1).

CEDS isolates were allocated to a distinct serovar, Bpyut, not exhibited by any other strain isolated during the study period. Additionally, all shared the same plasmid content, harbouring only the cryptic gonococcal plasmid, and showed highly similar PFGE-generated SpeI restriction patterns, 14 of them being identical. CEDS strains were also of the same origin, all submitted from the Venereal Disease Hospital located in Thessaloniki. They accounted for 63% of 27 viable gonococci that were received from this setting and represented 39% of all 43 gonorrhoea cases reported from Northern Greece during the 14 month period running from December 2006 through January 2008.

We conclude that CEDS gonococci were disseminated in Northern Greece by clonal spread of a single strain. Epidemiological data were obtained through a standard questionnaire answered by the patients, including demographic and sexual behaviour data, previous sexually transmitted disease history and area of acquisition of infection. No relation between the CEDS strains and any particular group of the population was apparent. Data were available for all CEDS strains apart from the first one isolated in December 2006. The second strain was isolated in the same month from a Spanish–English heterosexual contact, while the remaining 15 isolates were obtained after February 2008 from Greek heterosexual men who affirmed contacts with casual female partners in Thessaloniki.

Due to only slight reductions in susceptibility to cefotaxime, which is the cefalosporin regularly tested, CEDS strains passed unnoticed. As a consequence, patients were given the standard regimen used for gonorrhoea in the Venereal Hospital of Thessaloniki since 2002, which included cefuroxime sodium (single intramuscular dose of 1.5 g), plus tetracycline (100 mg twice daily for 8 days). Although therapeutic failures were not reported, the fact that the CEDS strains were subsequently found resistant to both agents does not guarantee this treatment’s outcome, and this could be a cause for the persistence of these strains.

The susceptibility profile of the CEDS cluster of gonococci resembles those of N. gonorrhoeae strains with reduced susceptibility to newer β-lactams reported previously from Japan1–3 and elsewhere.4,5 Cefalosporin susceptibility reductions have been associated with various types of mosaic penicillin-binding protein 2, occurring via homologous recombination between penA genes of N. gonorrhoeae and other Neisseria species.2,5

Most of the strains with decreased susceptibility to the newer cefalosporins reported so far, including CEDS in this

References


study, exhibited multidrug resistance indicating the operation of non-specific mechanisms, such as alterations in penR and mtrR genetic loci. Furthermore, strains with these characteristics are most often resistant to fluoroquinolones.\(^1\)\(^-\)\(^5\) Apart from its apparent clinical impact, the uniform multiply resistant pattern of cephalosporin non-susceptible strains from distant geographical areas suggests that they may have emerged from a limited number of strains, which were subsequently disseminated worldwide.\(^5\) Nevertheless, studies on isolates separated in time and place indicated a considerable degree of heterogeneity among gonococci with these characteristics.\(^5\)

For Greece, with established high rates of resistance to traditional anti-gonococcal agents and an estimated frequency of quinolone-resistant strains that reached a peak of 56% in 2007 (unpublished data of the NRCNG), the emergence of strains with diminished susceptibility to cephalosporins is threatening. From a global point of view as well, increasing reports of such non-susceptible gonococci since the 1990s is most alarming.

**Acknowledgements**

For their contributions to gonorrhoea surveillance, we thank Helen Avgerinou (Microbiology Laboratory of the ‘Andreas Sygros’ Hospital, Athens), Olga Paniara and Athena Argyropoulou (Microbiology Laboratory of the ‘Evangelismos’ General Hospital, Athens), Evangelos Papafragas and Maria Martsoukou (‘Sismanogleion’ General Hospital, Athens) and Alkiviadis Vatopoulos (National School for Public Health). We also thank Prof. Leonidas Tzouvelekis (Athens University Medical School) for helpful comments on the manuscript.

**Funding**

Surveillance of *Neisseria gonorrhoeae* antimicrobial susceptibility continuously contacted in the NRCNG is supported by the Hellenic Pasteur Institute.

**Transparency declarations**

None to declare.

**References**


Susceptibility of 170 isolates of the USA300 clone of MRSA to macrolides, clindamycin and the novel ketole cethromycin

Vicki Ann Luna¹*, Ze-Qi Xu², David A. Eiznhamer², Andrew C. Cannons¹ and Jacqueline Cattani¹

¹Center for Biological Defence, College of Public Health, University of South Florida, 3602 Spectrum Boulevard, Tampa, FL 33612, USA; ²Advanced Life Sciences, 1440 Davey Road, Woodridge, IL 60517, USA

Keywords: MIC, ketolide, antimicrobial activity

*Corresponding author. Tel: +1-813-974-3873; Fax: +1-813-974-1479; E-mail: vluna@health.usf.edu

Sir,

Historically, methicillin-resistant Staphylococcus aureus (MRSA) has been regarded as a nosocomial pathogen responsible for severe toxin-mediated disease or invasive pyogenic infections. In recent years, however, community-associated MRSA (CA-MRSA) has been reported from around the world. Although the first CA-MRSA strains were susceptible to most antimicrobials, antimicrobial resistance has increased. One CA-MRSA clone, identified by the CDC as pulsotype USA300, has been implicated in outbreaks within the USA and is resistant to many currently marketed antimicrobial agents.¹ This clone carries both the SCCmec IV element and the Panton–Valentine leukocidin (PVL) locus. The aim of this study was to compare the activity of the cethromycin cethromycin (formerly ABT-773), with potentially improved pharmacodynamic and therapeutic benefits, three other macrolides (azithromycin, clarithromycin and erythromycin) and one lincosamide (clindamycin) against the 170 USA300 isolates that are in the Center for Biological Defense (CBD) collection.

Seventeen MRSA isolates were procured from two hospital laboratories in Seattle, WA, USA. These were isolated from cultures of blood, skin lesions and tissue abscesses of patients seen in their clinics or emergency department in 2003 and 2004. Of the remaining 153 MRSA, 121 isolates were obtained from cultures of lesions, abscesses and nasopharyngeal specimens from outpatients seen at west central Florida clinical laboratories and doctor’s offices, whereas 32 MRSA were isolated from lesions, abscesses, nasopharyngeal and blood specimens acquired from patients seen at hospitals in the Tampa Bay area from 2004 to 2006. These isolates were previously characterized as USA300 by PFGE using Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA) standards for comparison.²

Cethromycin (obtained from Advanced Life Sciences, Woodridge, IL, USA) was prepared and diluted with calcium-adjusted Mueller–Hinton broth (Remel, Lenexa, KS, USA) using 96-well microtiter plates following standard protocols.³ The MIC and MBC were determined using orthodox procedures.³,⁴ Clarithromycin, erythromycin and clindamycin were tested with Sensititre® by TREK Diagnostic Systems (Cleveland, OH, USA), whereas azithromycin, erythromycin and clindamycin were tested using Etest® (AB Biodisk, North America Inc., Piscataway, NJ, USA), according to manufacturer’s instructions. All MIC interpretations followed CLSI guidelines.³ Clindamycin resistance was examined further with the D-test.⁵ Controls were S. aureus ATCC 29213, S. aureus ATCC BAA977 and S. aureus ATCC BAA976.

Cethromycin was effective against all 170 isolates (Table 1). The cethromycin MIC range was ≤0.002–0.125 mg/L, whereas the MIC₉₀ and MIC₉₀ were both ≤0.002 mg/L. The cethromycin MBC range was ≤0.002–1.0 mg/L, with the MBC₉₀ of ≤0.002 mg/L and the MBC₉₀ of 0.008 mg/L. Of the 170 CA-MRSA, 169 (99.4%) were intermediate or resistant to at least one macrolide. In contrast, 164 (96.5%) isolates were susceptible to clindamycin. Only six (3.5%) isolates demonstrated either constitutive resistance [4 (2.4%)] or inducible resistance [2 (1.2%)] to clindamycin. This is unlike the USA300 isolates in Boston where 57% of the isolates were clindamycin-resistant.¹ Against 10 erythromycin-susceptible isolates, the cethromycin MIC and MBC ranges were ≤0.002–0.008 mg/L, compared with the MIC range of ≤0.002–0.25 mg/L and MBC range of ≤0.002–1.0 mg/L obtained with the erythromycin-resistant isolates. However, MIC₉₀ and MBC₉₀ values, as well as MIC₉₀ and MBC₉₀ values, did not change regardless of erythromycin susceptibility. There were six isolates resistant to both erythromycin and clindamycin, against which cethromycin MIC and MBC ranges were 0.002–0.06 mg/L.

This population’s susceptibility to cethromycin is especially important in the light of the fact that most isolates were resistant to one or more macrolides. This collection of MRSA USA300 isolates was overwhelmingly macrolide-resistant and clindamycin-susceptible. The study of Chavez-Bueno et al.⁶ demonstrated a similar pattern in CA-MRSA isolates among children in Texas where the inducible resistance to clindamycin was slowly replaced by clindamycin susceptibility (non-inducible resistance). Most of the CA-MRSA isolates in the Texas study carried the erm(C) gene, with others carried erm(A) or msr(A) with or without erm(C).⁵ This phenotypic change suggests an alteration in the proportion of the macrolide resistance determinants in the population. Tenover et al.⁷ reported the msr(A) gene in isolates comprising a specific clone (USA300-0114). Accordingly, we hypothesized that most of the CBD collection of MRSA USA300 isolates (only one being USA300-0114) also carried a different determinant, putatively the msr(A)/msr(B) gene encoding an efflux protein that normally renders a bacterial cell resistant only to macrolides and streptogramin B compounds (MS phenotype) instead of the MLS₉ resistance genes [i.e. erm(A) and erm(C)]. Using the oligonucleotides described by Sutcliffe et al.,⁸ for PCR primers and a well-characterized MRSA strain NARSA 384 (USA300-0114) as positive control, we performed a PCR assay for the msr(A) gene on DNA extracted from all 170 isolates. After the amplicons were electrophoresed on a 1% agarose gel, a positive PCR reaction yielded a DNA band of 399 bp. All negative and questionable results were performed three times. The assay produced a positive reaction in 160 (94.1%) isolates, whereas only 10 (5.9%) of the 170 MRSA yielded negative PCR reactions. These