SUSCEPTIBILITY OF TOXIGENIC AND NON-TOXIGENIC *CORYNEBACTERIUM DIPHTHERIAE* ISOLATED IN 1997-2002 TO COMMONLY PRESCRIBED ANTIMICROBIALS: A CAUSE FOR CONCERN?

R. S. Kozlov[1] M. Warner[2] C. Kelly[3] M. Sukhorukova[1] A. Efstratiou[3]

1 Institute of Antimicrobial Chemotherapy, Smolensk State Medical Academy, P.O. Box 57, 28 Krupskaya Street, Smolensk, 214019, Russian Federation; 2 Antibiotic Resistance Monitoring & Reference Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK; 3 Health Protection Agency, Specialist and Reference Microbiology Division, Respiratory & Systemic Infection Laboratory, 61 Colindale Avenue, London NW9 5HT, UK

Abstract

Diphtheria re-emerged in countries of the former USSR in the 1990s with over 170,000 cases and 4,000 deaths reported to WHO during 1990-1998. The disease still remains endemic in many countries of South-East Asia, Africa and South America. The epidemiology of *Corynebacterium diphtheriae* is changing, as there has been much documentation of significant increases in the number of both invasive and non-invasive diseases caused by non-toxigenic strains. WHO currently recommends penicillin as the drug of choice and erythromycin as an alternative for patients with diphtheria and their close contacts. Macrolide resistance in corynebacteria has been previously documented. The susceptibility to penicillin G, amoxicillin/clavulanate, erythromycin, clindamycin, telithromycin and levofloxacin was determined by the agar dilution method on 89 toxigenic and non-toxigenic *C. diphtheriae* isolated from patients during the period 1997-2002. *C. diphtheriae* biotype *mitis* NCTC 11397, *Staphylococcus aureus* ATCC 29213, ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were used as controls. In comparison by MIC_{90}, telithromycin was the most active agent (MIC_{90} = 0.004 mg/L), followed by erythromycin (MIC_{90} = 0.015 mg/L), clindamycin (MIC_{90} = 0.125 mg/L), levofloxacin (MIC_{90} = 0.125 mg/L), penicillin G (MIC_{90} = 0.25 mg/L) and amoxicillin/clavulanate (MIC_{90} = 0.5 mg/L). Penicillin G and erythromycin retain their activity against *C. diphtheriae* and should be considered as first choice drugs in patients with diphtheria and close contacts. Telithromycin, clindamycin, levofloxacin and amoxicillin/clavulanate demonstrated favourable *in vitro* activity and may be considered as the alternatives based upon relevant data from clinical trials.

Introduction

In has been noted previously, that the epidemiology of infections caused by *C. diphtheriae* is changing over the time with significant increases in the number of both invasive and non-invasive diseases caused by non-toxigenic strains. In addition to the increase of incidence of infections, problem of antimicrobial resistance substantially complicated available approaches to the treatment. Diphtheria cases are usually treated by antitoxin as well as antibiotics, and antimicrobials are used to prevent dissemination of bacteria and toxin production, to avoid infection of contacts and for eradication of the organism in asymptomatic carriers. The drugs of choice recommended by the World Health Organization are penicillin and erythromycin. Other macrolides, penicillins, rifampicin, fluoroquinolones were suggested as alternative antibiotics for the treatment of diphtheria Documented studies on the *in vitro* activity of antimicrobials against *C. diphtheriae* are limited. Resistance to penicillin has not been documented in the peer-reviewed literature, however there have been some reports of «resistance» to -lactams published mostly in non-peer-reviewed, non-English medical literature, causing notable concern amongst clinicians. Since 1973, there were also reports on resistance of corynebacteria to macrolides and lincosamides, which re-emphasises the necessity to provide monitoring of antimicrobial resistance of *C. diphtheriae* not only to -lactams and macrolides, but also to possible alternative agents.

Objective

To determine the antimicrobial resistance of clinical strains of *C. diphtheriae* isolated in adults and children in different countries.

Materials & Methods

A total of 89 toxigenic and non-toxigenic *C. diphtheriae* isolated from patients in the NIS countries of the former USSR, including Russia (Smolensk, Smolensk region, Kaliningrad and Saint Petersburg), Belarus (Minsk and other regions), Kazakhstan, UK (London, Salisbury, Swansea) and Finland during the period 1997-2002 (Fig. 1) were included in this study.

Fig. 1. Centers from which C. diphtheriae strains were selected

Minimal inhibitory concentrations (MICs) of penicillin G, amoxicillin/clavulanate, erythromycin, clindamycin, telithromycin and levofloxacin were determined by agar dilution method. Plates were incubated for 24 h at 35°C at ambient air. *C. diphtheriae* biotype *mitis* NCTC 11397, *Staphylococcus aureus* ATCC 29213, ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were used as controls.

Results

Susceptibility testing results are presented in the Table below.

Table. MIC_{50}, MIC_{90} and MIC ranges of tested antimicrobials for C. diphtheriae

Antimicrobial	MIC_{50}, mg/L	MIC_{90}, mg/L	MIC range, mg/L
Penicillin G	0.25	0.25	0.125-0.25
Amoxicillin/clavulanate	0.25	0.5	0.125-0.5
Erythromycin	0.016	0.016	0.008-0.016
Clindamycin	0.125	0.125	0.125-0.125
Telithromycin	0.004	0.004	0.002-0.004
Levofloxacin	0.125	0.125	0.125-0.25

In comparison by MIC_{90}, telithromycin was the most active *in vitro* agent (MIC_{90} = 0.004 mg/L), followed by erythromycin (MIC_{90} = 0.015 mg/L), clindamycin (MIC_{90} = 0.125 mg/L), levofloxacin (MIC_{90} = 0.125 mg/L), penicillin G (MIC_{90} = 0.25 mg/L) and amoxicillin/clavulanate (MIC_{90} = 0.5 mg/L).
The MICs distributions of penicillin G, amoxicillin/clavulanate, erythromycin, clindamycin, telithromycin and levofloxacin for *C. diphtheriae* are presented on Fig. 2-7.

Fig. 2. MIC distribution of penicillin G

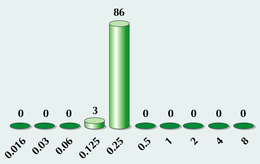


Fig. 3. MIC distribution of amoxicillin/clavulanate

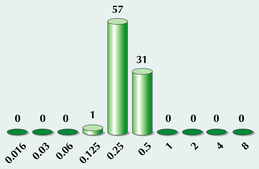


Fig. 4. MIC distribution of erythromycin

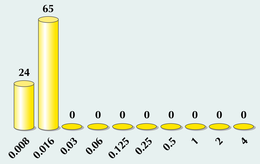


Fig. 5. MIC distribution of clindamycin

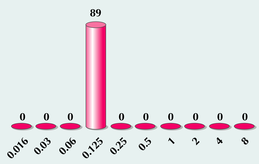


Fig. 6. MIC distribution of telithromycin

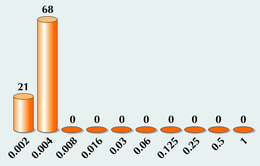
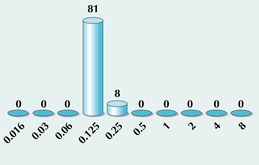


Fig. 7. MIC distribution of levofloxacin



Discussion & Conclusions

There are no internationally accepted susceptibility/resistant breakpoints for corynebacteria. However, analysis of the MIC distribution for tested antimicrobials and a comparison with previously published data suggested that there were not any substantial changes in MICs of isolates in this study in comparison to wild isolates. Such data therefore, allows us to make the following conclusions.

- Penicillin G and erythromycin retain their activity against *C. diphtheriae* and should be considered as a first choice in patients with diphtheria and close contacts.
- Telithromycin, clindamycin, levofloxacin and amoxicillin/clavulanate demonstrated favourable *in vitro* activity and might be used as the alternatives based upon relevant data from clinical trials.

Acknowledgements

- This work was performed within the remit of INTAS Project 01-2289 «Study of the role of non-toxigenic *Corynebacterium diphtheriae* in the epidemiology of diphtheria».
- Dr. Roman S. Kozlov is very grateful to FEMS Subcommittee on Congress Attendance Grants for awarding INTAS junior grant in order to attend the 1 FEMS Congress.