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In vitro antimicrobial activity of benzalkonium chloride against clinical isolates of *Streptococcus agalactiae*

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***Objectives*: Despite antibiotic prophylaxis for at-risk mothers during labour and delivery, *Streptococcus agalactiae* (group B *Streptococcus*; GBS) still causes substantial morbidity and mortality among newborns. In addition to the well-known side effects of the administration of antibiotics, resistance to drugs recommended for penicillin-allergic pregnant women, such as erythromycin and clindamycin, has increased, thus raising concern about the possibility of inadequate prophylaxis. On this basis we evaluated the antimicrobial activity of benzalkonium chloride against GBS, which has been described as an antimicrobial agent for the topical treatment of vaginal infections.**

***Methods*: A total of 52 GBS from pregnant women have been studied. The capacity of benzalkonium chloride as well as of penicillin, erythromycin, clindamycin, vancomycin, chloramphenicol and tetracycline to inhibit GBS was evaluated using broth macrodilution and microdilution methods, respectively.**

***Results*: While all the strains were penicillin- and vancomycin-susceptible, 19.2% were resistant to both erythromycin and clindamycin. In contrast, all GBS isolates were either inhibited or killed by benzalkonium chloride at not only low but also very similar concentrations (MIC_{90} = 3.12 mg/L).**

***Conclusions*: Benzalkonium chloride might represent an alternative strategy that is useful in reducing vaginal GBS colonization in pregnant women before delivery by topical treatment.**

Keywords: *S. agalactiae*, GBS, MICs, prophylaxis

Introduction

Streptococcus agalactiae (group B *Streptococcus*; GBS) infection has long been recognized as a frequent cause of morbidity and mortality in newborn infants.[1] Life-threatening complications of GBS bacteraemia, such as endocarditis, meningitis and fatal septicaemia with multiorgan failure, have been described over the last decades and GBS infections are a leading cause of neonatal mortality.[2,3] Approximately 10–30% of pregnant women are colonized in the vaginal or rectal area and it is from this source that most infections in the parturient emanate.[4] The revised Centers for Disease Control and Prevention guidelines issued in 2002 recommend a culture-based screening for vaginal-rectal colonization with GBS for all pregnant women at 35–37 weeks of gestation for prevention of early-onset GBS disease.[5] Antibiotic prophylaxis represents an important means to prevent perinatal disease. However, antibiotic failure or the side effects related to the antibiotic administration may contribute to persistent disease. In this context, benzalkonium chloride has been demonstrated to possess antimicrobial activity against different bacteria and its therapeutic role in vulvovaginal infections has been studied.[6] The present study was undertaken in order to evaluate the inhibitory effect of benzalkonium chloride on the growth of *S. agalactiae*.

Materials and methods

A total of 52 strains of GBS isolated from vaginal swabs of pregnant women screened for vaginal colonization were studied. According to CDC guidelines, all the specimens were inoculated onto CNA blood agar plates and soon after submerged in selective Todd–Hewitt broth (bioMérieux, France). After 4–5 h the broth was subcultured onto the same selective blood agar and all the plates were incubated for 18–24 h at 35°C in a candle jar.[7] Both β-haemolytic and non-haemolytic suspected colonies were identified as *S. agalactiae* by a latex agglutination test (bioMérieux, France).

In order to evaluate the capacity of benzalkonium chloride to interfere with GBS growth either the MIC or the MBC was determined by broth macrodilution method according to the NCCLS.[8]

The benzalkonium chloride powder was a gift from ACEF S.p.A, Piacenza, Italy. The stock solution (1 mg/mL) was prepared in warmed

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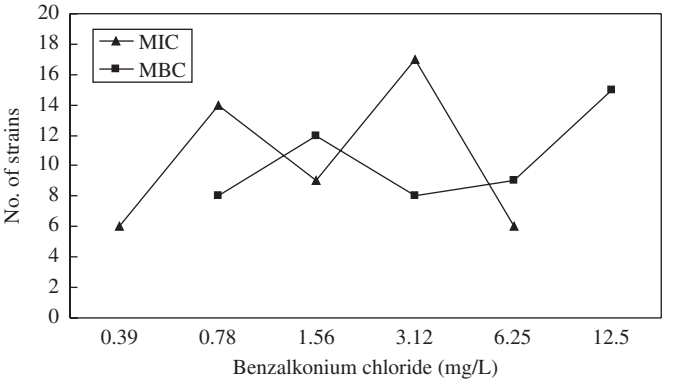

Figure 1. *In vitro* MIC and MBC of benzalkonium chloride against 52 strains of *Streptococcus agalactiae*.

distilled water and frozen in aliquots at –20°C. Serial twofold dilutions of the stock solution (0.048–50 mg/L) were prepared in 1 mL of Mueller–Hinton broth containing 2% laked horse blood. Each bacterial isolate was suspended in 5 mL of sterile saline and vortexed. The bacterial suspension, adjusted to a turbidity equivalent to that of a 0.5 McFarland standard, was then diluted 1:100 with broth and distributed among the tubes. A broth-tube free of benzalkonium chloride was added as a growth control. The final concentration of the inoculum was $\sim 5 \times 10^5$ cfu/mL. After 24 h of incubation at 37°C, the MIC value was recorded as the lowest concentration of benzalkonium chloride that inhibited visible growth when compared with that in the control growth tube. After this reading the incubation was prolonged until 48 h to verify a putative time-related effect. MBC was determined by inoculating 100 µL of broth taken at 24 h of incubation from tubes without microbial growth onto blood agar plates. After incubation for 24 h at 37°C in a candle jar, the cfu were counted and MBC was defined as the lowest concentration of benzalkonium chloride resulting in the death of 99.9% or more of the initial inoculum. Furthermore, in order to detect a possible interaction of the culture medium with benzalkonium chloride, the same procedure was repeated using two different types of media (i.e. thioglycolate and trypticase soy broth).

The antibiotic susceptibility profiles were determined for all the bacterial isolates. In particular, chloramphenicol, clindamycin, erythromycin, penicillin, tetracycline and vancomycin were tested using the microdilution method according to the NCCLS guidelines.[8]

Results

When the capacity of benzalkonium chloride to interfere with GBS growth was evaluated, all the isolates tested were inhibited at MIC values ranging between 0.39 and 6.25 mg/L. The MIC_{90} (that inhibited 90% of the strains) was 3.12 mg/L. The MBC values ranged between 0.78 and 12.50 mg/L and were similar or slightly higher than the MIC values (Figure 1). Neither prolonged incubation (up to 48 h) nor the use of different culture media interfered with the benzalkonium chloride antibacterial activity (data not shown).

With regard to the antibiotic susceptibility pattern of the bacteria tested, all the GBS isolates were susceptible to penicillin, vancomycin and chloramphenicol. Out of 52 strains 40 (77%) were resistant to tetracycline, 10 (19.2%) to erythromycin and 12 (23%) were resistant to clindamycin. In particular, all the erythromycin-resistant strains were also resistant to both clindamycin and tetracycline.

Finally, results clearly show that for all the strains tested benzalkonium chloride susceptibility is not related to the antibiotic resistance.

Discussion

GBS continues to be an important cause of maternal and neonatal morbidity.[3] In pregnant women the current treatment strategy to prevent early-onset neonatal diseases is limited to intrapartum antibiotic prophylaxis. GBS is uniformly susceptible to penicillin *in vitro*, and penicillin G is the drug of choice when the diagnosis is established. However, an increased resistance to erythromycin and clindamycin, the drugs of choice for women with serious penicillin allergy, has been observed.[9] Antibiotic prophylaxis requires careful assessment of the epidemiology of GBS disease and close surveillance of susceptibility patterns. In this regard, 19.2% of GBS tested were resistant to erythromycin, clindamycin and tetracycline. These results are consistent with those reported by Manning *et al*.[9] Although the strains were penicillin-susceptible, this finding raises concern about the possibility of inadequate prophylaxis using currently recommended alternatives in penicillin-allergic patients. In addition, the use of late prenatal cultures might be impractical because of the possible lack of their prompt availability.[10] Finally, it should be kept in mind that the antibiotic treatment may be responsible for a significant variation in the indigenous bacterial flora of women.

On the basis of these considerations, it appears that it is of importance to evaluate the possibility to reduce GBS colonization by using products other than antibiotics. The notion that benzalkonium chloride has been used as an antimicrobial agent for topical treatment and prevention of vaginal infections[6] prompted us to evaluate its antibacterial activity against GBS. Our *in vitro* experiments show that GBS strains are both inhibited and killed at similar benzalkonium chloride concentrations. These antimicrobial activities against GBS are achieved with concentrations lower than that of most commercially available topical products.

In conclusion, the above considerations indicate that benzalkonium chloride may represent an alternative strategy useful to reduce vaginal GBS colonization in pregnant women before delivery, although its efficacy and safety must be validated by means of consistent clinical trials.

Transparency declarations

None to declare.

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