

Streptococci from acute otitis media and their treatment by enterocins. By A. Lauková and A. Kandričáková. *Institute of Animal Physiology Slovak Academy of Sciences, Šoltésovej 4-6, 04001 Košice, Slovakia*

Introduction

Many streptococcal species are part of the normal commensal non-pathogenic microflora of the mouth, skin, intestine, and upper respiratory tract of humans. However, some of them can cause infections in humans or animals. In children, acute otitis media (AOM) is one of the most frequently occurring infection⁽¹⁾. Among bacterial agents responsible for this infection *Streptococcus pneumoniae* and *Str. pyogenes* from A group of streptococci are the most often detected⁽²⁾. In general, streptococci represent Gram-positive bacteria belonging to the phylum Firmicutes, to the family Streptococcaceae and to the genus Streptococcus. By streptococci caused AOM can be standardly treated with oral administration of antibiotics. However, there is problem with an increased resistance of microbiota to antibiotics, streptococci including. Without antibiotic treatment, a bacterial acute otitis can cause e.g. sudden perforation of the eardrum. From the basic research point of view, formerly mentioned resistance problem lead us to search for new alternative way to solve this problem. Therefore, we decided to test sensitivity of AOM streptococci to enterocins. Enterocins (Ent) represent ribosomally synthesized proteinaceous antimicrobial substances with inhibitory activity against Gram-positive and/or Gram-negative bacteria which are produced mostly by the species *Enterococcus faecium*⁽³⁾. Our Laboratory of Animal Microbiology (LAM) have been focused on enterocins study for years. Enterocins have been successfully applied to reduce spoilage bacteria in different animal and/or food⁽⁴⁻⁶⁾. Therefore, the aim of this study was to test sensitivity of *Str. pyogenes* and *Str. pneumoniae* isolated from children AOM to Ents produced by the strains *E. faecium* of different origin (our isolates). This aim was conducted to look for the antimicrobial spectrum of our enterocins to indicate new way for reduction of former indicated problems as well as to show further possibilities for enterocins use.

Material and Methods

Str. pyogenes (7) and *Str. pneumoniae* (8) from AOM were kindly supplied by Dr. Hupková and Dr. Bukovský (Comenius University, Bratislava). Semi-purified enterocins (Ent EM 41, Ent EM 42, Ent M, Ent 55, Ent 412, Ent 2019, Ent M3a, Ent 4231, Ent A,P) were used in the study. They are produced⁽³⁾ by the *E. faecium* strains of different origin, isolated at our LAM (Slovak Academy of Sciences, Košice, Slovakia). Ents EM 41, EM 42 are produced by *E. faecium* EM41 and EM42 from ostrich; producer strains of Ent M and Ent A (P) are environmental *E. faecium* AL41 and CCM7419-EK13; *E. faecium* EF2019-CCM 7420 from rabbit faeces produces Ent 2019; *E. faecium* M3a from rabbit meat produces Ent M3a; Ent 55 is produced by avian *E. faecium* EF 55 and Ent 412 is produced by *E. faecium* EF 412 from faeces of horses. Enterocins were semi-purified according to previously reported protocols⁽⁷⁻⁹⁾. Ents were with initial activity was as follows: Ent 412, Ent 55, Ent A (P), Ent 2019 25 600 AU/ml; Ent M, Ent M3a 6 400AU/ml; Ent EM 41 12 800 AU/ml against the principal isolate EA 5 strain (our isolate from piglet). Ent EM42 showed initial activity 800 AU/ml against *Str. pneumoniae* Spn47. Sensitivity of streptococci to Ents was tested by the quantitative agar spot test⁽¹⁰⁾ using Brian Heart agar (Becton & Dickinson, Cockeysville, USA). Inhibitory activity

was expressed in Arbitrary units per milliliter (AU/ml); it responds to the highest dilution of Ent causing the inhibition of the growth of the indicator strain. As positive control was used *E. avium* EA5 (isolate from piglets, the most sensitive indicator strain).

Results and Discussion

Among 8 strains of *Str. pneumoniae*, 7 were sensitive at least to 1 Ent (Table 1). *Str. pneumoniae* SPn 754 was sensitive to 5 Ents (activity 100-800 AU/ml-Table 1). Among 7 strains of *Str. pyogenes*, 5 were sensitive to Ents.. The most active was Ent A (P); it inhibited the growth of 3 *Str. pneumoniae* and 4 *Str. pyogenes* (100-3200 AU/ml-Table 1). The other Ents inhibited the growth of 4 to 6 strains (100-3 200 AU/ml), excepted Ent EM 41, Ent M and Ent M3a to which only 2 respectively 1 indicator strain were/was sensitive. Although lower count of the growth of *Str. pyogenes* was inhibited, they were more sensitive to Ents because higher activity (in Arbitrary Units) was measured comparing with *Str. pneumoniae*; except SPn 58; there was noted inhibitory activity 3200 AU/ml, although the strain was sensitive only to 3 Ents. In medicine, the mostly used bacteriocin is lantibiotic (bacteriocin) nisin. It was used e.g. to prevent dermatological lesions as well as gingivitis⁽¹¹⁾. Even eradication of *Helicobacter pylori* was reported by Blackburn and Projan by nisin⁽¹²⁾. Moreover, bacteriocin-lantibiotic produced by e.g. *S. gallinarum* TU 3928⁽¹³⁾ can be used to treat abscess or dermatitis; its effect is comparable with effect of erythromycin. On the other hand, in veterinary medicine enterocins and/or their producer strains have found better utilization and they were used with beneficial effect in poultry infected with *Salmonella* ^(6,14) and/or in rabbits⁽¹⁵⁾. Results achieved in our study possess pioniering character because enterocins were not tested against streptococci isolated from AOM yet. Therefore, our results represent a new view for enterocins use. Directly, besides resistance to ATB, e.g. during 2001-2006 years, 92 children with AOM were hospitalized and more than 60% were unsuccessfully treated by antibiotics, among those 55% were children up to age 1 year⁽¹⁶⁾. Lauková and Šedová ⁽¹⁷⁾ found sensitivity of *Pseudomonas aruginosa* strains isolated from sanitary hospital sources and theatres to Ents EK13= (A,P), M, 2019, 55.

Conclusion

In spite of preliminary results which of course request the additional studies, tendency of Ents to inhibit streptococci isolated from AOM was shown. Our results are indication to continue in this strategy in connection with the beneficial use of proteinaceous substances in medicine.

Acknowledgement

The work was supported by the project VEGA No. 2/0004/14 and the project no. 26220220152 (implementation supported by the Reserch and Development Operational Programme funded by the ERDF). We are gratefull to Mrs. Margita Bodnárová for her skillfull laboratory work and we thank to Dr. Hupková and Dr. Bukovský (Comenius University, Bratislava, Slovakia) for their kindness to provide us the strains. Partial part of these reuslts was presented at 7th International Symposium on Anaerobic Microbiology

ISAM2011, p.82as Abstract in Conference Book-ISBN978-80-968618-6-6 and published in Acta Microbiologica et Immunologica Hungarica 59 (1), 2012, p. 21-27.

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Table 1 Treatment of *Streptococcus pyogenes* and *Str. pneumoniae* strains isolated from children otitis media acuta by enterocins (expressed in Arbitrary units per ml, AU/ml)

Streptococci	Ent EM 41	Ent EM 42	Ent M	Ent 55	Ent 412	Ent 2019	Ent M3a	Ent A(P)
SPn 47	-	800	-	-	-	-	-	-
SPn 49	100	100	-	-	-	-	100	100
SPn 57	-	100	-	-	-	-	-	-
SPn 58	-	-	-	-	3200	3200	-	100
SPn 60	-	100	-	-	-	-	-	-
SPn 922	-	100	-	-	-	-	-	-
SPn. 754	-	-	100	100	100	800	-	400
SP 111	100	-	-	-	-	100	-	-
SP 114	-	-	-	100	-	100	-	400
SP 115	-	-	-	1600	-	-	-	200
SP 116	-	-	-	400	400	800	-	3200
SP 117	-	-	-	400	100	100	-	3200

SPn-*Streptococcus pneumoniae*, SP-*Streptococcus pyogenes*, Initial activity of enterocins: Ent 412, Ent 55, Ent A (P), Ent 2019 25 600 AU/ml; Ent M, Ent M3a 6400AU/ml; Ent EM 41 12 800 AU/ml. EM 42 was with initial activity 800 AU/ml. The strains SP 112, SP 113, SPn46 were not sensitive to Ents used. Activity is expressed in AU/ml.