Polish Journal of Microbiology
2005, Vol. 54, No 1, 63–67

Potential Possibilities of Using Phage Typing in Elimination of Multidrug Resistant Staphylococci

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Received 26 August, received in revised form 22 November 2004, accepted 30 November 2004

Abstract

Coagulase-negative staphylococci (CoNS) have become the most often isolated bacteria from blood culture, spinal fluid and respiratory tracts of neonates. These nosocomial strains are often resistant to oxacillin and other antibiotics (macrolides, aminoglycosides and fluorochinolones). 50 multidrug resistant CoNS strains isolated from bloodstream neonatal infections were tested for sensitivity to 23 lytic staphylococcus bacteriophages. No lytic patterns for 19 of the phages were observed. Phages P4, A3R and 676/Z were active against 46%, 54% and 56% of the strains, respectively. In general, 60% of CoNS isolates were susceptible to one or more of the staphylococcus bacteriophages.

Key words: staphylococci, phage therapy

Neonatal Intensive Care Unit's (NICU) patients seemed to be the most susceptible group to bloodstream infections. Intensive treatment using arterial, intravenous and broviac catheters, ventilation and other risk factors such as a low birth weight, and neutropenia, favour neonatal nosocomial infections (Beck-Saque *et al*., 1994; Huebner and Kropec, 1995; Reimer *et al.*, 1997; Rupp and Archer, 1994). Coagulase-negative staphylococci (CoNS) have become the most often isolated bacteria from blood culture, spinal fluid and respiratory tracts of neonates (Burnie *et al*., 1997; Drulis-Kawa *et al*., 2002; Huebner and Kropec, 1995; Kellogg *et al*., 2000; Pfaller *et al*., 1998; Villari *et al*., 2000; Weinstein *et al*., 1998). Prolonged period of hospitalisation and unrestricted use of antibiotics leads to the development of antibiotic resistance and to nosocomial infection problems (Beck-Saque *et al*., 1994; Hall, 1991; Pfaller *et al*., 1998). From 60% to 90% of CoNS strains isolated from bloodstream infections are reported as resistant to oxacillin. These strains are also frequently resistant to other antibiotics (macrolides, aminoglycosides and fluoro-chinolones) (Drulis-Kawa *et al*., 2002; Pfaller *et al*., 1998; von Eiff *et al*., 2000). Because glycopeptides are often the only drugs active against multi-resistant gram-positive strains, the emergence of low sensitivity to vancomycin was also reported (Hiramatsu *et al*., 1997; Sieradzki *et al*., 1999; Smith *et al*., 1999). Therefore, new antibiotics or antimicrobial agents are needed to treat neonatal infections and to decrease selection of multidrug-resistant nosocomial pathogens (Baquero, 1997; Biswas *et al*., 2002; Sulakvelidze *et al*., 2001; von Eiff *et al*., 2000).

The aim of this study was to determine the sensitivity spectra of CoNS strains isolated from neonatal bloodstream infections to antibiotics and to specific bacteriophages.

A collection of a total of 50 CoNS strains was isolated from blood culture and tracheal aspirates of the neonatal patients treated in Neonatal Intensive Care Units (NICU) in Wroclaw, Poland. Isolates were included only from patients with monomicrobic infection. The examined collection of bacteria contained 32 *S. epidermidis* isolates, 11 *S. haemolyticus* isolates and 7 other CoNS *Staphylococcus* isolates (*S. simulans*

[n = 3], *S. chromogenes* [n = 2], *S. warneri* [n = 1], *S. xylosus* [n = 1]). The sensitivity to antimicrobial agents was measured by applying the following antibiotics: oxacillin, gentamicin, chloramphenicol, clindamycin, azithromycin, ciprofloxacin, tetracycline, and vancomycin. MICs of the antimicrobial agents tested were performed using E-tests (AB Biodisc, Sweden). Detection of oxacillin/ methicillin resistance was carried out on Mueller Hinton agar (MHA) + 2% NaCl. Direct colony suspensions in saline to a 0.5 units of McFarland turbidity scale were plated and incubated at 35°C in ambient atmosphere for 48 hours. Detection of glycopeptide resistance of strains was carried out on Brain Heart Infusion agar (BHI). Colonies from a 24 hour blood plate were suspended in BHI broth to a turbidity of 2 in McFarland scale. E-tests were incubated at 35°C in ambient atmosphere for a first reading after 24 hours and confirmed after 48 hours. Detection of resistance to other antibiotics was carried out on the MHA plates using 10^8 cell ml^{-1} suspension in saline (0.5 McFarland scale). E-tests were incubated at 35°C in ambient atmosphere for 16–20 hours. Interpretation of susceptibility to antibiotics was determined according to the NCCLS standards [NCCLS 2000]. The breakpoints of the susceptibility (resistance) were for: oxacillin ≤0,25 μg ml^{-1} (≥0,5 μg ml^{-1}); gentamicin ≤4 μg ml^{-1} (≥16 μg ml^{-1}); chloramphenicol ≤8 μg ml^{-1} (≥32 μg ml^{-1}); clindamycin ≤0,5 μg ml^{-1} (≥4 μg ml^{-1}); azithromycin ≤2 μg ml^{-1} (≥8 μg ml^{-1}); ciprofloxacin ≤1 μg ml^{-1} (≥4 μg ml^{-1}); tetracycline ≤4 μg ml^{-1} (≥16 μg ml^{-1}); vancomycin ≤4 μg ml^{-1} (≥32 μg ml^{-1}). As a quality control the *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 14990 strains were used.

23 staphylococcal bacteriophages with wide spectrum of activity have been used. The phages came from the Collection of the Bacteriophage Laboratory of the L. Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw. The phage stocks were prepared by standard methods and the results of typing were recorded as previously (Ślopek, 1972).

In vitro susceptibilities of CoNS strains to tested antibiotics are reported in Table I. Ninety six percent of the isolates showed high level of resistance to oxacillin (MICs ≥128 μg ml^{-1}). High rates, 86% of resistance to gentamicin (MICs ≥16 μg ml^{-1}) and azithromycin (MICs ≥8 μg ml^{-1}), and 60% of resistance to ciprofloxacin (MICs ≥4 μg ml^{-1}) were also observed. Low level of resistance (8%, 34%, and 38%) was noticed in case of tetracycline, chloramphenicol and clindamycin, respectively. All of the CoNS bacteria were sensitive to vancomycin. Among the 23 bacteriophages used for phage typing only four showed lytic activity to isolated staphylococci (Table II). Phage P4 lysed twenty-three of the 50 tested strains. The lytic activity against 28 and 27 of the 50 isolates was shown by phage 676/Z and A3/R, respectively. *S. epidermidis* ATCC 14990 was also sensitive to these phages. Only one of tested strains was sensitive to phage A5/L. In general, 60% of isolated CoNS strains were susceptible to one or more of the staphylococcus bacteriophages.

Table I

In vitro activities of eight antibiotics against coagulase-negative staphylococci (n = 50) isolated from neonatal blood culture and tracheal aspirate

Antibiotics	No of isolates inhibited by MIC [μg ml^{-1}] of:											Susceptibility	
	≤ 0,25	0.5	1	2	4	8	16	32	64	128	≥ 256	% S[a]	% R[b]
Oxacillin	2	0	0	0	0	0	0	0	0	3	45	4	96
Gentamicin	5	1	0	0	1	0	1	6	5	8	23	14	86
Chloramphenicol	0	0	1	5	25	1	1	1	1	1	14	64	34
Clindamycin	27	2	2	0	0	0	0	2	0	0	17	58	38
Azithromycin	1	6	0	0	0	0	1	3	7	4	28	14	86
Ciprofloxacin	17	1	1	1	0	1	1	28	NT[c]	NT	NT	38	60
Tetracycline	2	5	32	7	0	0	0	0	1	1	2	92	8
Vancomycin	0	0	0	0	50	0	0	0	0	0	0	100	0

[a] – S; susceptible; [b] – R; resistant; [c] – NT; concentration not tested

The high risk of bloodstream infections in neonatal ICU forces the use of antibiotics, most often beta-lactam on account of their low toxicity. Therefore gram-positive as well as gram-negative nosocomial strains developed various mechanisms of resistance to beta-lactam antibiotics. The number of methicillin or oxacillin resistant coagulase negative staphylococcus strains (MRCNS) in various hospital wards is different. In the study of Antimicrobial Surveillance Program (SENTRY) carried out in United States and Canada

Table II
In vitro activities of four *S. aureus* bacteriophages against coagulase-negative staphylococci isolated from neonatal blood culture and tracheal aspirate

Species	No. of strains susceptible to bacteriophage				No. of strains susceptible to one or more phages
	P4	676/Z	A5/L	A3/R	
S. epidermidis (n = 32)	13	18	1	19	20
S. haemolyticus (n = 11)	4	4	0	2	4
S. simulans (n = 3)	2	2	0	2	2
S. chromogenes (n = 2)	2	2	0	2	2
S. warneri (n = 1)	1	1	0	1	1
S. xylosus (n = 1)	1	1	0	1	1
Total (n =5 0)	23	28	1	27	30
S. epidermidis ATCC 14990 (control)	0	1	0	1	

68% of *S.epidermidis* isolated from bloodstream infections were oxacillin-resistant (Pfaller *et al*., 1998). In our previous study (Drulis-Kawa, 2002) 90% of *S.epidermidis* isolated from neonatal bloodstream infections were resistant to oxacillin. In our investigation we stated nearly the same high number of MRCNS strains (96%). This means that the use of beta-lactam antibiotics against the most frequent neonatal pathogens is strongly limited. The CoNS isolates tested in this study were also resistant to other useful antibiotics such as aminoglycosides (amikacin, gentamicin, netilmicin) 86%, macrolides (erythromycin, azithromycin) 86% and lincosamides (clindamycin, lincomycin) 38%. The tetracycline resistance of tested staphylococci amounted to 8%, but because of its side effects tetracycline can not be used to treat children. The most effective antimicrobial agent against CoNS strains has become vancomycin (glycopeptide), because almost none of the isolated staphylococci strains are resistant to this antibiotic (Drulis-Kawa, 2002; Pfaller *et al*., 1998). It has been already reported that *S. haemolyticus* strains and other CoNS clinical strains have showed heterogeneous expression of teicoplanin resistance (glycopeptide) so it could be associated with heterogeneous resistance to vancomycin (Sieradzki *et al*., 1999). Efficacy of vancomycin for treatment of methicillin-resistant staphylococcus bloodstream infection decreased when vancomycin MICs for MRS isolates were 1–2 μg ml^{-1} (Sakoulas *et al*., 2004). It means that we should look for alternative antibiotics or antimicrobial agents to treat the multidrug resistant staphylococcus infections.

The idea of using bacteriophages in bacterial indentification and infections treatment is well known. In 1925 Sonnenschein used phages as diagnostic reagents for *Salmonella paratyphi* B and *Salmonella typhi* strains. Different bacterial species may be subdivided into phage types with identical phage sensitivity by using the method of phage typing (Richardson *et al*., 1999; Ślopek *et al*., 1972; Ślopek *et al*., 1973; Zawieja *et al*., 1986). The method of phage typing of coagulase-negative staphylococci with phage sets for human and animal staphylococcal strains has been used in many countries: Poland (Heczko *et al*., 1977), Georgia (Akatov *et al*., 1982), Germany (Holmberg, 1978), Spain (Martin-de-Nicolas *et al*., 1990), Netherlands (Verhoef *et al*., 1972), United States (Skahan and Parisi, 1977), Hungary (Barcs *et al*., 1994) and Denmark (Jarlov 1999). Total bacteriophages typability was between 35–58% (Skahan and Parisi, 1977) and 76% (Barcs *et al*., 1994). The susceptibility of bacterial clinical isolates to the bacteriophages showed considerable geographic variation and new combinations set of CoNS phages increase typability (Talbot and Parisi, 1976). Rosdahl noticed (Rosdahl *et al*., 1990) that antibiotic resistant staphylococci, especially MRS, were rarely typable (11–13%) in comparison to susceptible strains (36–50%). In our study we have found similar dependence, because among 23 lytic staphylococcus phages only four were active against multidrug resistant strains.

The most detailed historical publications documenting phage therapy have come from Stefan Ślopek's group (Ślopek *et al*., 1981a, b; Ślopek *et al*., 1984). In 518 of the infection cases (the patients ranged in age from 1 week to 86 years) phage therapy was used following unsuccessful treatment with all available antibiotics. Number of successful phage treatment ranged from 75% to 100% (92% overall). The successful phage treatment was also showed in therapy of bacterial infections in cancer patients and in therapy for antibiotic-resistant septicemia in man (Weber-Dąbrowska *et al*., 2003; Weber-Dąbrowska *et al*., 2000; Weber-Dąbrowska *et al*., 2001). The efficiency of phage treatment in various staphylococcus infections was described also by other authors (Sakandelidze and Meipariani, 1974; Sulakvelidze *et al*., 2001; Zhukov-Verezhnikov

et al., 1978). In presented study 60% of the multidrug resistant staphylococci isolated from severe neonatal infections were sensitive to lytic action of four bacteriophages. While the most of the isolates occurred high rates of resistance to vancomycin (MIC 4 μg ml^{-1}) we suppose that phage therapy could be useful as supportive or alternative treatment staphylococci infections in neonates.

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