Cross-transmission rates of enterococcal isolates among newborns in a neonatal intensive care unit

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Abstract

Enterococci are important pathogens causing nosocomial infections and patients at risk include also premature babies requiring intensive care treatment. Our aim was to assess occurrence and cross transmission rates of enterococci among neonatal patients of a hospital ward during a two months period. Rectal and skin samples were taken between day one and 60 of infants' age. Colonization with various potentially pathogenic bacteria was correlated with developing a subsequent infection. Enterococcal isolates were identified by colony morphology. The bacterial species was assessed and antibiotic susceptibilities were determined. A molecular analysis of 20 investigated enterococcal isolates revealed prevalence of commensal strain types; hospital-associated strain types or multi-resistant variants were absent. Cross transmission of *E. faecium* and *E. faecalis* isolates among neonatal patients attending the intensive crare unit at the same time was demonstrable. Introduction of hospital-associated, multi-resistant variants into this special setting has to be avoided to reduce the risk of subsequent infections.

Introduction

Rates of enterococcal infections among patients in intensive care increased dramatically in the last two decades worldwide.[1] Enterococcal isolates (*Enterococcus faecalis* and *E. faecium*) are ranked as the second most important nosocomial pathogen in intensive crare unit (ICU) acquired bloodstream infections in Europe.[2] Infections occur in severely ill and immuno-compromised patients revealing also premature babies and newborns at risk for acquiring enterococcal infections.[3,4] Transmission rates of enterococcal isolates are high among nosocomial patients, even higher than for *S. aureus* and *E. coli*.[5] Increased importance of enterococci especially *E. faecium* as a nosocomial pathogen is linked to a preferred prevalence of hospital-associated strain types that possess acquired antibiotic resistance properties, specific molecular markers and clonal types identifiable by molecular typing techniques such as Multi-locus sequence typing (MLST).[6] On the other hand, commensal clonal types of *E. faecalis* and *E. faecium* exhibit important constituents of the healthy human intestinal flora and are among the first and early colonizers of the infants' intestines.[7] In the present study we investigated rectal (and skin) colonization, cross-transmission rates and clonal types of enterococci among newborns of a neonatal ICU of a German hospital over a period of two months.

Brief Report

From April to May 2006, 20 patients attending a German neonatal ICU were screened for bacterial rectal and skin colonizations. All newborns were admitted to a separate ward and were kept aside from other pediatric patients. Samples were taken in relation to clinical signs of infection of the babies (fever, etc.) or when mothers had risk factors or infections (amnion infection, vaginal group B streptococcus colonization, etc). Potentially pathogenic bacteria such as *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterobacteriaceae* and *Enterococcus* spp. were assessed. Five patients were rectally screened consecutively, two and three times, respectively (Table 1). Rectal and skin samples were primarily screened on two non-selective media; Brilliance UTI Clarity agar (Oxoid/Thermo Fischer Scientific, Wesel, Germany) and Trypticase Soy-Agar + 5% Sheep Blood (bioMerieux, Nuertingen, Germany). Morphologically different isolates per sample were further identified by Vitek® 2 (bioMerieux, Nürtingen, D). All but three enterococcal isolates were available for a subsequent analysis. Antibiotic susceptibilities for enterococci were initially determined by VITEK® 2 (bioMérieux, according to CLSI criteria and later confirmed by an in house microbroth dilution method.[8] Epidemiological markers to differentiate hospital-associated strains of *E. faecium* from colonizing variants were determined by PCR as described recently (*esp, hyl_{Efm}, IS*16)..[9,10] To investigate clonal relatedness of strains macrorestriction analysis in Pulsed-Field Gel Electrophoresis was performed as described[9] and subsequent analysis was done using a Dice coefficient and UPGMA clustering (BioNumercis v. 5.1; Applied Maths, Belgium). Multi-locus sequence typing (MLST) was performed to differentiate between commensal and hospital-associated strain types as given elsewhere (*http://efaecium.mlst.net/ and http://efaecalis.mlst.net/*).

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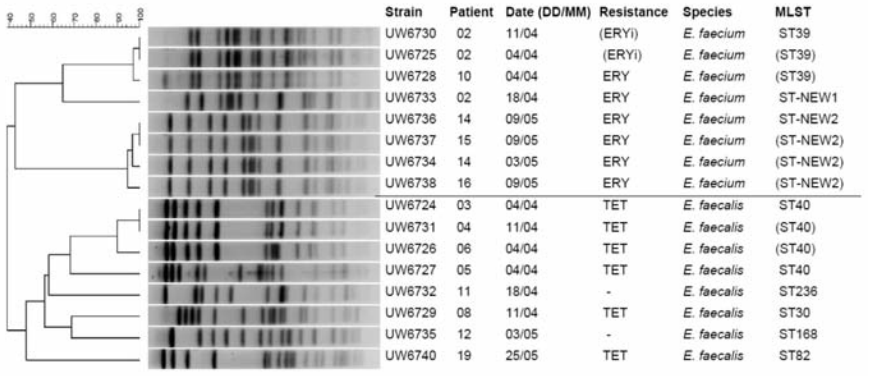
Results

Of the 20 screened neonatal patients only two samples did not grow bacteria at all (of the genera and species described above; Table 1). Ten neonatal patients showed signs of an infection and received immediate antibiotic treatment (Table 1). Screening samples of 18 patients grew enterococci and from 14 children isolates were accessible. From two patients consecutive isolates were analyzed (Figure 1). Altogether eight *E. faecalis* and eight *E. faecium* isolates were available for a molecular analysis. Antibiotic susceptibility testing revealed a very small spectrum of acquired resistances; mainly erythromycin (*E. faecium*) and tetracycline (*E. faecalis*; Figure 1) representing widespread acquired resistance characteristics also prevalent among animal, environmental and human colonizing, commensal enterococcal strains. Molecular markers of hospital-associated *E. faecium* strains (*esp,*

Table 1. Microbiological results of the rectal screenings of all neonatal intensive care unit patients during a two-months' period. Only potentially pathogenic bacteria of the genera *Staphylococcus, Enterococcus, Acinetobacter* and the family Enterobacteriaceae were assessed.

Date (DD/MM)	Patient No.	Age at admission (days)	Enterococcus	Other bacteria	Infection / Sepsis	Gestational age in weeks (+days)	Birth weight (g)	Clinical situation (child)	Antibiotic treatment	Remarks
04/04	1	0	No	No	No	38	2.920	Only monitoring		
04/04	2	0	Yes	*Escherichia coli*	No	31 (+2)	1.175	Apnea, bronchopulmonary	Premature infant dysplasia	
11/04			Yes	*E. coli, Streptococcus agalactiae*	No					
18/04			Yes	*E. coli*	No					
04/04	3	7	Yes	*Klebsiella oxytocha; Staphylococcus epidermidis*	No	39	3.110	Galactosaemia		
04/04	4	0	No	*S. epidermidis*	No	40	3.960	Asphyxia		Mother: spontaneous cardiac arrest during birth
11/04			Yes	*E. coli,* MRSE	No					
04/04	5	0	Yes	*E. coli,* MRSE	Yes	28 (+0)	740	Sepsis, anaemia, hyperbilirubinaemia, respiratory distress syndrome, bronchopulmonary dysplasia	AMP/GEN VAN/CTX	Premature infant
11/04			Yes*	*E. coli,* MRSE						
04/04	6	0	Yes	*E. coli, S. epidermidis*	No	41	4.360	Hypertrophia, hyperglobulia		
11/04	7	0	No	*E. coli*	Yes	40	4.330	Hypertrophia, perinatal infection	AMP/GEN	
11/04	8	0	Yes	*E. coli*	Yes	40	3.320	Perinatal infection	AMP/GEN PIP/GEN	
11/04	9	0	No	No	No	35 (+2)	2.245	Respiratory distress syndrome		Premature infant
18/04			Yes*	No	No					
11/04	10	0	Yes	*E. coli,* MRSE	No	38	3.820	Unknown syndrome with micrognathia and apnea		
18/04	11	3	Yes	*E. coli, S. epidermidis, Proteus mirabilis*	Yes	41	3.670	Perinatal infection	AMP/GEN	*P. mirabilis* from stomach aspirate
25/04	12	4	Yes	*E. coli*	Yes	38	3.610	Omphalitis, conjunctivitis	AMP/GEN ECO	
25/04	13	3	Yes*	*Acinetobacter spp.*	No	40	2.410	Ventriculum septum defect		Bacterial growth from nasal swab
03/05	14	0	Yes		Yes	32 (+0)	1.970	Hypoglycaemia, respiratory distress syndrome, perinatal infection	Mother: MTR, AMP, CXM Baby: AMP/GEN; CTX	Premature infant
09/05			Yes	*E. coli*						
09/05	15	0	Yes	MRSA, MRSE	(Yes)	32 (+2)	1.640	Respiratory distress syndrome, Ductus omphalomesentericus	AMP/GEN	Premature infant; initial antibiotic therapy terminated after 3 days
09/05	16	0	Yes	*S. epidermidis, Enterobacter cloacae*	Yes	41	3.560	Perinatal infection, conjunctivitis	AMP/CTX ECO	Bacterial growth from ear swab
09/05	17	0	Yes	*E. coli*	Yes	38	3.545	Perinatal infection, respiratory distress syndrome	AMP/CTX	
09/05	18	2	Yes	*S. epidermidis, E. cloacae*	Yes	40	4.150	Omphalitis, perinatal infection	AMP/GEN CTX	
25/05	19	0	Yes	*S. epidermidis*	No	35	3.100	Pneumonia		Bacterial growth from skin swab; viral pneumonia

MRSE, Methicillin-resistant *Staphylococcus epidermidis*; MRSA, Methicillin-resistant *Staphylococcus aureus*; AMP, ampicillin; CTX, cefotaxime; CXM, cefuroxime; ECO, Ecolicin (Erythromycin); GBS+, group B Streptococcus-positive; GEN, gentamicin; MTR, metronidazole; PIP, piperazillin; VAN, vancomycin. * These isolates were not available for a subsequent analysis.



Strain	Patient	Date (DD/MM)	Resistance	Species	MLST
UW6730	02	11/04	(ERYi)	*E. faecium*	ST39
UW6725	02	04/04	(ERYi)	*E. faecium*	(ST39)
UW6728	10	04/04	ERY	*E. faecium*	(ST39)
UW6733	02	18/04	ERY	*E. faecium*	ST-NEW1
UW6736	14	09/05	ERY	*E. faecium*	ST-NEW2
UW6737	15	09/05	ERY	*E. faecium*	(ST-NEW2)
UW6734	14	03/05	ERY	*E. faecium*	(ST-NEW2)
UW6738	16	09/05	ERY	*E. faecium*	(ST-NEW2)
UW6724	03	04/04	TET	*E. faecalis*	ST40
UW6731	04	11/04	TET	*E. faecalis*	(ST40)
UW6726	06	04/04	TET	*E. faecalis*	(ST40)
UW6727	05	04/04	TET	*E. faecalis*	ST40
UW6732	11	18/04	-	*E. faecalis*	ST236
UW6729	08	11/04	TET	*E. faecalis*	ST30
UW6735	12	03/05	-	*E. faecalis*	ST168
UW6740	19	25/05	TET	*E. faecalis*	ST82

Figure 1. *Sma*I macrorestriction patterns in Pulsed-Field Gel Electrophoresis (PFGE) of all enterococcal isolates colonizing neonatal patients. Clusters of related isolates from samples of different neonates suggest horizontal transmission between patients (clonal spread), for instance, isolates *E. faecalis* UW6724 [Patient no. 3], UW6731 [Patient no. 4], and UW6726 [Patient no. 6]. MLST types in parentheses are predicted according to an identical PFGE profile. Profiles ST-NEW1 and ST-NEW2 are new MLST types due to new (N) allele sequences which have the following allelic profiles: ST-NEW1 [N-8-8-N-6-27-6]; ST-NEW2 [25-8-N-17-10-N-6] (see also http://efaecium.mlst.net/ and http://efaecalis.mlst.net/). ERY(i), erythromycin (intermediate); TET, tetracycline; MLST, Multi-locus sequence typing; ST, sequence type (of MLST); n.d., not determined.

hyl_{Efm}, IS16) were absent in the eight *E. faecium* isolates. MLST results confirmed prevalence of commensal *E. faecium* strain types among the investigated patients (Figure 1). Four isolates of *E. faecalis* revealed MLST strain type ST40 known to be highly prevalent among various ecological sources and which is also isolated from severe invasive infections like endocarditis and sepsis[11] and from cases of bovine mastitis (Zischka and Werner, unpublished data). However, hospital-associated strain types belonging to clonal complexes CC2 and CC9 were not identified among the *E. faecalis* isolates. Cross transmission between the newborn babies attending the neonatal ICU at the same time period was demonstrated for *E. faecium* and *E. faecalis* isolates as well as ongoing colonization with identical strain types in single neonates (Figure 1).

Conclusions

A microbiological and molecular analysis of the enterococcal isolates from stool colonizations in newborn infants of a neonatal ICU revealed prevalence of colonizing strain types. Hospital-associated strain types or multi-resistant variants were not identified. Nevertheless, also colonizing strain types could cause endogenous enterococcal infections; however, the pathogens causing the described invasive infections could not been identified here. Cross transmission of enterococcal isolates among newborns attending the neonatal ICU at the same time was shown. To elucidate the source of these early colonizing isolates, an independent and comprehensive follow-up study including stool sampling and subsequent analyses from babies' mothers and medical staff is planned.

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