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Orally administered colistin leads to colistin-resistant intestinal flora and fails to prevent faecal colonisation with extended-spectrum β -lactamase-producing enterobacteria in hospitalised newborns

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ABSTRACT

Colonisation and infection with extended-spectrum β -lactamase-producing Enterobacteriaceae (ESBL-E) is an emerging problem. The aim of this study was to investigate whether colistin, which is reported to be effective against multiresistant enterobacteria, prevents ESBL-E colonisation in neonates. For prophylaxis of necrotizing enterocolitis, oral gentamicin (15 mg/kg/day) is routinely used in all neonates hospitalised at the Neonatal Intensive Care Unit of University Hospital Graz (Austria). During the study period from May 2005 to September 2007, three ESBL-E outbreaks (total duration 18 months) occurred. During these outbreaks, gentamicin was immediately replaced by oral colistin (8 mg/kg/day) in all hospitalised neonates. All neonates colonised with ESBL-E during the study period were retrospectively analysed with regard to the influence of colistin on ESBL-E colonisation. Genetic relatedness of isolates was assessed by repetitive sequence-based polymerase chain reaction (rep-PCR). During the study period, 30 (4.5%) of 667 neonates were colonised with ESBL-E. Twelve of twenty-one patients colonised with *Klebsiella pneumoniae* (ESBL-Kp) and one of nine patients colonised with *Klebsiella oxytoca* (ESBL-Ko) had received oral colistin at time of colonisation with ESBL-E. Among ESBL-Kp, the rate of colistin resistance was significantly higher in the colistin group ($P = 0.0075$). Four different clones of ESBL-Kp and three different clones of ESBL-Ko were isolated, indicating the occurrence of patient-to-patient transmission. Colistin-resistant as well as colistin-susceptible isolates were detected within the same clones, indicating induction of resistance. At the dosage used, oral colistin did not prevent colonisation with ESBL-E and appeared to select colistin-resistant strains or to induce colistin resistance.

1. Introduction

Extended-spectrum β -lactamase-producing Enterobacteriaceae (ESBL-E) are of growing importance both in community and healthcare settings. As well as being resistant to penicillins and cephalosporins, these bacteria often show additional antimicrobial resistances (including aminoglycoside resistance) and have been associated with increased morbidity and mortality, especially in critically ill patients [1]. Intestinal colonisation with bacteria, including Enterobacteriaceae, is believed to be a co-factor in the pathogenesis of neonatal necrotising enterocolitis (NEC) [2,3], and ESBL-producing *Klebsiella pneumoniae* has been implicated in at least one outbreak of NEC [4]. Enteral aminoglycosides [3] are used in some institutions as prophylaxis against NEC. However, the increasing prevalence of ESBL-E that are resistant to aminoglycosides means that the utility of other antibiotic classes requires further evaluation. Colistin has been reported to be effective in the treatment of infections with ESBL-E in adults and children [5–7], in selective digestive decontamination (SDD) [8] and in NEC prophylaxis [9].

During three outbreaks of colonisation with ESBL-E in the Neonatal Intensive Care Unit (NICU) of the University Hospital Graz (Graz, Austria), oral colistin was used in place of gentamicin as prophylaxis against NEC. Here we describe a retrospective analysis of the impact of this intervention.

2. Methods

2.1. Setting and study design

At the NICU of the University Hospital Graz, a combination of gentamicin [7.5 mg/kg every 12 h (q12h) orally], nystatin [10 000 IU/kg every 6 h (q6h)] and enteral probiotics [*Lactobacillus rhamnosus* GG 0.5 g (0.5×10^9 colony-forming units) q12h] for all hospitalised neonates during the whole stay is the local standard of care for preventing NEC [10]. Furthermore, stool cultures from all NICU patients are routinely taken twice a week for active surveillance of intestinal colonisation. Patients colonised with ESBL-E are cohorted with dedicated staff, and hygienic precautions are intensified by wearing gloves and gowns. Since the majority of isolated ESBL-E were observed to be resistant to gentamicin, NEC prophylaxis was modified from May 2005 to September 2007; oral gentamicin was replaced by oral colistin (2 mg/kg q6h) in all NICU patients as long as ESBL-E colonisation was detected in at least one NICU patient.

To evaluate the ability of orally administered colistin to prevent colonisation with ESBL-E, all neonates colonised with ESBL-E during the study period were analysed retrospectively.

2.2. Laboratory examinations

Faecal samples were inoculated into brain–heart infusion broth (Becton Dickinson, Cockeysville, MD) and were incubated at 35 °C overnight and then streaked onto three plates, including blood agar, MacConkey agar and chromID ESBL agar (bioMérieux, Marcy l'Etoile, France). Identification of all Gram-negative bacilli was

performed by standard methods, and susceptibility testing for antimicrobial agents was performed and interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) [11] by means of a semi-automated system (VITEK II instrument; bioMérieux) as well as by the disk diffusion test. ESBL production was detected by the resistance phenotype, the CLSI double-disk synergy test and Etest using ESBL confirmation strips. Susceptibility testing for colistin and gentamicin of ESBL-E strains was additionally performed by Etest (AB BIODISK, Solna, Sweden). For colistin, a minimum inhibitory concentration (MIC) breakpoint of ≤ 2 mg/L was applied for susceptibility [11,12].

To assess clonality, molecular typing of ESBL-E isolates was performed by semi-automated repetitive sequence-based polymerase chain reaction (rep-PCR) on a DiversiLab system (bioMérieux) as described previously [13]. Clonality was defined as similarity $>95\%$.

2.3. Statistical analyses

Data are presented as median and range or as counts and proportions. Fisher's exact test was used to compare proportions.

3. Results

From May 2005 to September 2007, three outbreaks with clustered occurrence of intestinal ESBL-E colonisation and subsequent replacement of gentamicin by colistin as part of NEC prophylaxis were observed (May–September 2005, April–December 2006 and June–September 2007). No ESBL-E colonisation was observed beyond

these outbreaks. During the outbreaks, 30 term and preterm born neonates (gestational age 24–40 weeks, median 31 weeks; birth weight 540–3700 g, median 1780 g; 14 female; age at colonisation 3–102 days, median 22 days) among 667 hospitalised patients (4.5%) had been colonised with ESBL-producing *K. pneumoniae* (ESBL-Kp) ($n = 21$) or *Klebsiella oxytoca* (ESBL-Ko) ($n = 9$). Twelve of twenty-one patients colonised with ESBL-Kp and one of nine patients colonised with ESBL-Ko had received colistin orally prior to colonisation with ESBL-E (colistin group, $n = 13$) instead of gentamicin (gentamicin group, $n = 17$). In one patient in the colistin group gentamicin had been switched to colistin 3 days before the patient was colonised with ESBL-Kp; all other patients in the colistin group received colistin from the first day of their hospitalisation.

All ESBL-Ko and 11 (52.4%) of 21 ESBL-Kp isolates were colistin-susceptible. Moreover, 3 (25%) of 12 ESBL-Kp isolates in the colistin group and 8 (88.9%) of 9 ESBL-Kp isolates in the gentamicin-group were susceptible to colistin ($P = 0.0075$). All but two isolates (one ESBL-Ko in the gentamicin group and one ESBL-Kp in the colistin group) were gentamicin-resistant (Table 1).

Four different clones of ESBL-Kp (clusters of ten and six isolates as well as two single isolates) and three different clones of ESBL-Ko (clusters of four and two isolates as well as one single isolate) were identified, indicating the occurrence of patient-to-patient transmission in the majority of patients. Five isolates (three ESBL-Kp and two ESBL-Ko) were not available for molecular typing. Interestingly, within the two major clones of ESBL-Kp, colistin-susceptible (six and two isolates, respectively) and colistin-resistant (four isolates each) isolates were detected.

In the analysed period, no infections clearly attributable to ESBL-E and no adverse effects of colistin were observed.

4. Discussion

Owing to the high rate of gentamicin resistance among ESBL-E, colistin was administered instead of gentamicin during ESBL-E outbreaks. However, nearly one-half of analysed patients were colonised with ESBL-E despite receiving colistin prophylaxis. Therefore, colistin was abandoned as part of NEC prophylaxis after September 2007.

The percentage of colistin resistance in isolated ESBL-Kp was significantly higher in the colistin group compared with the gentamicin group. Oral administration of colistin might therefore select colistin-resistant isolates or induce colistin resistance during the short period between intestinal colonisation and detection of ESBL-E [5]. This might explain the detection of colistin-susceptible as well as colistin-resistant ESBL-Kp isolates within the same clone. On the other hand, in four patients colistin-susceptible ESBL-E (three ESBL-Kp and one ESBL-Ko) were isolated despite colistin administration. Because colistin susceptibility testing of consecutive isolates after ESBL-E colonisation was not routinely performed, it was not possible to evaluate the development of resistance in primarily susceptible strains. There are two main possible explanations for colistin failing to prevent colonisation with colistin-susceptible ESBL-E. First, the faecal concentration of colistin might be too low to prevent colonisation effectively. Second, colistin might have been inactivated by stool and therefore lack effectiveness [14]. There are two main limitations of this retrospective study that must be taken into account when interpreting the results.

First, as reported in the literature [1], patient-to-patient transmission was the major mode of ESBL-E acquisition. Since colistin was only used during outbreaks, patients in the colistin group were certainly at higher risk of colonisation with ESBL-E by nosocomial transmission. Second, the same dose of colistin was administered in all patients. It might well be that higher colistin dosages and concentrations are more effective in preventing ESBL-E colonisation and avoiding the induction of resistance.

5. Conclusion

At the dosage used, oral administration of either gentamicin or colistin does not reliably prevent colonisation with ESBL-E in NICU patients. Moreover, it appears to induce antibiotic resistance or to select antibiotic-resistant bacteria. Randomised studies and further analyses are needed to assess the usefulness of colistin for SDD or NEC prophylaxis in settings with increased ESBL-E occurrence. Furthermore, development of resistance has to be taken into account when analysing the benefits and drawbacks of the widespread use of enteral antibiotics for prophylactic purposes.

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Competing interests

None declared.

Ethical approval

Not required.

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Table 1

Colistin and gentamicin susceptibility of isolated extended-spectrum β -lactamase-producing Enterobacteriaceae (ESBL-E). All patients received either colistin (colistin group) or gentamicin (gentamicin group) prior to colonisation with ESBL-E

Isolate	Susceptibility [<i>n</i> (%)]		
	Colistin group	Gentamicin group	Total
ESBL-producing <i>Klebsiella pneumoniae</i>	12	9	21
Colistin-resistant	9 (75) *	1 (11.1) *	10 (47.6)
Gentamicin-resistant	11 (91.7)	9 (100)	20 (95.2)
ESBL-producing <i>Klebsiella oxytoca</i>	1	8	9
Colistin-resistant	0 (0)	0 (0)	0 (0)
Gentamicin-resistant	1 (100)	7 (87.5)	8 (88.9)

* $P = 0.0075$, Fisher's exact test.