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ORIGINAL ARTICLE

Risk factors for acquisition of CTX-M-15 extended-spectrum beta-lactamase-producing Klebsiella pneumoniae during an outbreak in a neonatal intensive care unit in Norway

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Abstract

Background: A CTX-M-15 extended-spectrum beta-lactamase (ESBL)-producing Klebsiella pneumoniae was responsible for an outbreak in the neonatal intensive care unit (NICU) at Stavanger University Hospital, Norway over a 5-month period (November 2008–April 2009). The risk factors for acquiring ESBL-producing K. pneumoniae during the outbreak were examined in this study. *Methods:* Faecal or rectal cultures were obtained from infants hospitalized in the NICU during the outbreak period and examined for ESBL-producing K. pneumoniae. Data were retrospectively retrieved from the medical records, including sex, gestational age, birth weight, indwelling central vascular catheter, continuous positive airway pressure (CPAP), mechanical ventilation, parenteral nutrition, antibiotic treatment, mode of delivery (vaginal vs caesarean), length of hospital stay, and mortality. *Results:* A total of 216 infants were hospitalized in the NICU during the outbreak period, of whom 212 were screened; 51 (24%) scored positive for faecal colonization with ESBL-producing K. pneumoniae. One infant acquired a clinical infection. Forty-four colonized infants and 55 non-colonized infants were included in the risk analysis. Colonized infants had a lower birth weight, lower gestational age, and a longer hospital stay compared to non-colonized infants. By logistic regression, prematurity (gestational age <37 weeks) and treatment with antibiotics were independent risk factors for acquiring ESBL-producing K. pneumoniae in the final model. *Conclusion:* Prematurity and treatment with antibiotics were independent risk factors for colonization during this NICU outbreak with ESBL-producing K. pneumoniae.

Keywords: *Neonatal intensive care unit, outbreak, ESBL, Klebsiella pneumoniae, risk factors*

Introduction

Enterobacteriaceae, mainly Klebsiella pneumoniae, Escherichia coli, and Serratia marcescens, are responsible for the majority of reported bacterial nosocomial outbreaks in neonatal intensive care units (NICUs) [1]. Outbreaks due to Enterobacteriaceae have been associated with increased neonatal morbidity and mortality and increased length of stay and cost of hospitalization [2,3]. The emergence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae represents an even greater threat, with profound therapeutic implications [4]. Since first described in 1983, an alarming number of outbreaks and clusters due to ESBL-producing Enterobacteriaceae in NICUs have been reported, many due to Klebsiella species [2,5–9].

In response to the growing trend of outbreaks with multidrug-resistant Enterobacteriaceae, the identification of risk factors for colonization and infection have become essential for the adoption of preventive strategies. Several studies have shown that low gestational age, low birth weight, the use of invasive devices, antibiotic treatment, and the duration of hospital stay are associated with ESBL-producing Enterobacteriaceae colonization in NICUs, but only some of these studies have identified independent

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risk factors [5,6,10–12]. Furthermore, risk factors may vary considerably, which is attributed to differences in the organization of the NICUs, the level and standard of care, the prevalence of ESBL-producing Enterobacteriaceae in different populations, and local antibiotic guidelines and their use.

We recently described an outbreak due to CTX-M-15 ESBL-producing K. pneumoniae that occurred in the NICU at Stavanger University Hospital during the winter 2008–2009 [13]. This was to our knowledge the first reported NICU outbreak with ESBL-producing Enterobacteriaceae in Scandinavia. We performed a retrospective study to identify risk factors for acquiring ESBL-producing K. pneumoniae colonization during this NICU outbreak.

Methods

Setting

Stavanger University Hospital has a highly specialized 21-bed NICU and serves approximately 550 admissions and 7000 patient-days per year. The hospital has 5000 deliveries annually and serves as a secondary referral centre for a neighbouring hospital with 1500 deliveries. The NICU consists of 4 adjacent rooms, including an isolation unit. Excluding surgical patients, all neonates including premature infants from gestational age (GA) 23 weeks are treated in the unit.

Description of the outbreak

Details of the outbreak have been described previously [13]. Briefly, the outbreak was recognized when ESBL-producing K. pneumoniae were recovered in samples from 3 extremely premature infants. An outbreak investigation was initiated; all hospitalized infants and infants discharged during the previous 3 months were screened for ESBL-producing K. pneumoniae in faecal or rectal samples. To terminate the outbreak, a temporary and fully operative NICU was established within the hospital for a period of 2.5 months. An infant admitted from another hospital was identified as a probable index case. After the last colonized infants had been discharged, the original NICU was decontaminated and reopened. ESBL-producing K. pneumoniae were not isolated in the unit prior to the admission of the index case, and there have been no recurrences. Finally, we defined the outbreak period as starting 27 November 2008 and ending 17 April 2009.

As described previously [13], all ESBL-producing K. pneumoniae isolates typically expressed resistance to extended-spectrum cephalosporins and aztreonam, as well as clavulanic acid synergy. The outbreak strain was susceptible to carbapenems, ciprofloxacin, and tigecycline, but displayed co-resistance to nitrofurantoin, trimethoprim–sulfamethoxazole, tobramycin, and gentamicin. Sequence analysis revealed $bla_{CTX\text{-}M\text{-}15}$ in 58 faecal isolates obtained from each of the colonized patients and the blood culture isolate from 1 infant. A total of 56/58 faecal isolates had indistinguishable pulsed-field gel electrophoresis (PFGE) patterns, designated type I, whereas 2 isolates showed unrelated type II patterns.

Definitions

Colonization was defined when ESBL-producing K. pneumoniae were isolated in faecal or rectal samples from infants without clinical symptoms or signs of infection. Infection was defined when ESBL-producing K. pneumoniae were isolated from blood, urine, or cerebrospinal fluid from infants with symptoms or signs of infection. Prematurity was defined as GA $<$37 completed weeks.

Inclusion and exclusion criteria

Parents of all the colonized infants admitted to the original or temporary NICU during the outbreak period were invited to allow their infant to participate in the risk analyses for acquiring ESBL-producing K. pneumoniae. Infants who had the majority of their hospital stay at another hospital and infants colonized on the maternity wards were not included. Parents of all the non-colonized infants hospitalized in the original NICU were invited to allow their infant to serve as a control. Information regarding sex, gestational age, birth weight, indwelling central vascular catheter, continuous positive airway pressure (CPAP), mechanical ventilation, parenteral nutrition, antibiotic treatment, mode of delivery (vaginal vs. caesarean), length of stay, and death were retrieved retrospectively from the medical records of the infants included. The regional ethics committee and the Norwegian data inspectorate approved the study, and informed consent was obtained from the parents.

Statistical analysis

Continuous variables are presented as the median and quartiles, and binary variables as the count with percentage. Differences were analyzed with the non-parametric Mann–Whitney *U*-test and Pearson's Chi-square test for continuous and categorical variables, respectively. Possible associations between various risk factors and colonization with ESBL-producing K. pneumoniae analyzed by logistic

regression models, and odds ratios (OR) with 95% confidence intervals (CI), were calculated. Each variable was first entered separately into simple regression models. Covariates significant at the 10% level were included in backward stepwise logistic and linear regression analyses. Final models included the remaining covariates significant at the 5% level. All analyses were 2-tailed, and a p-level of <0.05 was considered statistically significant. Analyses were performed using SPSS for Windows version 18.0 (Chicago, IL, USA).

Results

During the 5-month outbreak period 132 infants were admitted to the original NICU and 84 to the temporary NICU. Of these, 212 (98.1%) infants were screened for ESBL-producing K. pneumoniae. Four infants hospitalized in the original NICU were lost to follow-up. In addition, 257 possible contacts on the maternity wards were screened. In total, 58 infants were colonized: 47 in the original NICU, 4 in the temporary NICU, and 7 contacts on the maternity wards. The rate of faecal colonization of ESBL-producing K. pneumoniae was 36.7% in the original NICU and 4.8% in the temporary NICU ($p<0.001$). One infant achieved a non-fatal severe sepsis due to ESBL-producing K. pneumoniae (1.7%).

Risk analyses

Two of the colonized infants hospitalized in the original NICU were transferred from another hospital where they spent the majority of their hospital stay and were not included in the risk analyses. Consent to participate and subsequent inclusion in the study was obtained for 44 of the remaining 49 colonized or infected children in the 2 NICUs and for 55 of the 81 non-colonized infants admitted to the original NICU. Forty-three of the participating colonized infants had PFGE type I, and 1 infant had PFGE type II.

Colonized infants were born at a lower GA, with lower birth weight, and had a longer duration of hospitalization compared to non-colonized infants (Table I). Table II shows the numbers of colonized and non-colonized infants grouped according to their GA. By univariate analyses, mechanical ventilation, treatment with CPAP, treatment with antibiotics, oxygen supplementation, total parenteral nutrition, indwelling catheter, prematurity, and length of stay were all risk factors for becoming colonized, whereas gender and mode of delivery were not (Table III). When prematurity and length of stay were analyzed together, only prematurity was a risk factor for colonization (data not shown). When analyzed in a multiple regression model, prematurity, mechanical ventilation, and antibiotic treatment were risk factors. In the final model, only antibiotic treatment and prematurity remained as independent risk factors for being colonized (Table III).

Table II. Numbers of infants colonized with extended-spectrum beta-lactamase-producing Klebsiella pneumoniae during an outbreak in a neonatal intensive care unit grouped by gestational age.

Gestational age	Colonized infants, *n* (%)	Non-colonized infants, *n* (%)
>37 weeks	8 (19)	35 (81)
32–37 weeks	21 (62)	13 (38)[a]
<32 weeks	15 (68)	7 (32)[a]

[a]$p<0.001$ compared to gestational age >37 weeks.

Discussion

A high rate of colonized infants (24%) was observed in this first outbreak due to ESBL-producing K. pneumoniae described in a Scandinavian NICU. Multivariate analysis revealed that prematurity and treatment with antibiotics were independent risk factors for being colonized.

Health care-associated outbreaks in NICUs represent a worldwide challenge, which is further complicated by the emergence of multidrug-resistant Gram-negative bacteria such as ESBL-producing Enterobacteriaceae. Antimicrobial resistance has become one of the world's most pressing threats to human health due to the rapid spread and persistence of resistance and lack of new antibiotics [14,15]. Targeted preventive measures and improved outcomes are dependent upon the identification of risk.

Table I. Characteristics of infants colonized with extended-spectrum beta-lactamase-producing Klebsiella pneumoniae or not during an outbreak in a neonatal intensive care unit.[a]

	Colonized infants ($n=44$)	Non-colonized ($n=55$)	p-Value
Gestational age (weeks)	34.2 (30.4–36.6)	38 (34.6–40.1)	<0.001
Birth weight (g)	2065 (1270–2849)	3018 (2170–3612)	<0.001
Hospitalization (days)	19 (11–68)	7 (3–16)	<0.001

[a]Results are given as median (quartiles).

Table III. Logistic regression models of risk factors for being colonized with extended-spectrum beta-lactamase-producing Klebsiella pneumoniae during an outbreak in a neonatal intensive care unit.

	Unadjusted model		Fully adjusted model		Final model	
Risk factors	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
Mechanical ventilation	18 (2.2, 146)	0.007	36 (1.2, 1059)	0.036		
CPAP	5.4 (2.2, 13.4)	<0.001	0.4 (0.04, 3.7)	0.4		
Treatment with oxygen	6.5 (2.6, 16.2)	<0.001	1.6 (0.3, 8.8)	0.6		
Treatment with antibiotics	5.8 (2.4, 14.5)	<0.001	6.6 (1.4, 31.6)	0.017	5.6 (2.1, 15.3)	0.001
Indwelling catheter	4.3 (1.2, 14.5)	0.02	0.05 (0.002, 1.5)	0.08		
Total parenteral nutrition	5.1 (1.8, 14.6)	0.002	0.7 (0.08, 7.9)	0.8		
Length of stay (days)	1.0 (1.0, 1.0)	0.001	1.0 (1.0, 1.0)	0.3		
Male gender	1.2 (0.6, 2.8)	0.6				
Caesarean section	2.2 (0.9, 5.0)	0.06				
Gestational age <37 weeks	7.9 (3.1, 20.2)	<0.001	7.3 (1.6, 33.6)	0.01	7.6 (2.8, 20.9)	<0.001
Gestational age <32 weeks	3.5 (1.3, 9.7)	0.014				

OR, odds ratio; CI, confidence interval; CPAP, continuous positive airway pressure.

Premature infants are susceptible to infections due to the relative immaturity of their immune systems, poor skin integrity, repeated invasive procedures, and exposure to numerous caregivers and an environment conducive to bacterial colonization [16]. In this outbreak, prematurity was an independent risk factor for colonization with ESBL-producing K. pneumoniae, even for those with GA 32–37 weeks. To our knowledge, this has not previously been demonstrated in a similar setting, but prematurity itself has been shown to be a risk factor for clinical infection in neonates colonized with ESBL-producing Enterobacteriaceae [17].

A large proportion of NICU patients will require antibiotics once or multiple times during a NICU stay, interfering with the establishment of a normal intestinal bacterial flora [16]. Despite a narrow antibiotic empirical regime with ampicillin and gentamicin for neonatal sepsis in our NICU, it increased the risk of colonization with ESBL-producing Enterobacteriaceae, as previously reported by others [10]. One possible mechanism could be co-resistance; K. pneumoniae is inherently resistant to ampicillin due to chromosomal SHV-1, and the outbreak strain was resistant to gentamicin. Secondly, colonization increases with the relative loss of the normal gastrointestinal flora [14,15]. Antibiotic treatment in general and the combination of cephalosporin plus aminoglycoside have been reported to be risk factors for colonization with ESBL-producing Enterobacteriaceae in neonates in other studies [5,6]. The rational use of antibiotics in the NICU should be reinforced with emphasis on good antimicrobial stewardship. Surveillance studies from different regions are essential for updating guidelines on empirical antimicrobial regimes in NICUs.

There was a notable difference in duration of hospitalization in colonized versus non-colonized infants. It seems logical that the duration of stay with prolonged exposure to a hospital environment is an independent risk factor for faecal colonization with ESBL-producing K. pneumoniae. Others have reported an association [5,10–12]. However, in our study the length of stay was not an independent risk factor when corrected for prematurity and other factors. The results from our study may indicate that the underlying condition of the infant and antibiotic treatment are more important than the duration of hospitalization for the risk of being colonized.

Interestingly, the rate of colonization was considerably lower in the temporary NICU compared to the original NICU (4.8% vs. 36.7%). The outbreak remained unrecognized for 2 months in the original NICU, allowing a large number of infants to become colonized. Awareness of the outbreak, cohorting in 2 separate NICUs, and an increased focus on hygiene measures appeared to play an important role in limiting the transmission of ESBL-producing K. pneumoniae among the infants. Transient hand carriage and transmission of ESBL-producing K. pneumoniae by health care workers in NICUs have previously been demonstrated [2,7,18,19]. Improved compliance with hand hygiene has been shown to be the single most important, yet the least expensive infection control activity in the NICU, and associated with a significant decrease in rates of nosocomial infections [16].

The present study was performed retrospectively, but all the analyzed variables were routinely recorded prospectively and systematically in the medical records and are therefore of the same quality as in a prospective study. The relatively small sample size may be a limitation for the detection of all independent risk factors for colonization. However, it is a strength that almost all infants hospitalized during the outbreak were tested for ESBL-producing

K. pneumoniae, and a high proportion of infants were included in the study.

In conclusion, our study has shown that prematurity and treatment with antibiotics may be independent risk factors for being colonized during an outbreak with CTX-M-15 ESBL-producing K. pneumoniae in a NICU. The result may provide a basis for preventive measures and targeted interventions during similar outbreaks.

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Declaration of interest: The authors have no conflicts of interest.

References

[1] Cipolla D, Giuffre M, Mammina C, Corsello G. Prevention of nosocomial infections and surveillance of emerging resistances in NICU. J Matern Fetal Neonatal Med 2011;24 (Suppl 1):23–6.

[2] Abdel-Hady H, Hawas S, El-Daker M, El-Kady R. Extended-spectrum beta-lactamase producing Klebsiella pneumoniae in neonatal intensive care unit. J Perinatol 2008;28: 685–90.

[3] Stone PW, Gupta A, Loughrey M, Della-Latta P, Cimiotti J, Larson E, et al. Attributable costs and length of stay of an extended-spectrum beta-lactamase-producing Klebsiella pneumoniae outbreak in a neonatal intensive care unit. Infect Control Hosp Epidemiol 2003;24:601–6.

[4] Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill 2008;13.pii:19044.

[5] Pessoa-Silva CL, Meurer Moreira B, Camara Almeida V, Flannery B, Almeida Lins MC, Mello Sampaio JL, et al. Extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in a neonatal intensive care unit: risk factors for infection and colonization. J Hosp Infect 2003;53:198–206.

[6] Cassettari VC, da Silveira IR, Dropa M, Lincopan N, Mamizuka EM, Matte MH, et al. Risk factors for colonisation of newborn infants during an outbreak of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in an intermediate-risk neonatal unit. J Hosp Infect 2009; 71:340–7.

[7] Dashti AA, Jadaon MM, Gomaa HH, Noronha B, Udo EE. Transmission of a Klebsiella pneumoniae clone harbouring genes for CTX-M-15-like and SHV-112 enzymes in a neonatal intensive care unit of a Kuwaiti hospital. J Med Microbiol 2010;59:687–92.

[8] Bagattini M, Crivaro V, Di Popolo A, Gentile F, Scarcella A, Triassi M, et al. Molecular epidemiology of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in a neonatal intensive care unit. J Antimicrob Chemother 2006;57:979–82.

[9] Rastogi V, Nirwan PS, Jain S, Kapil A. Nosocomial outbreak of septicaemia in neonatal intensive care unit due to extended spectrum beta-lactamase producing Klebsiella pneumoniae showing multiple mechanisms of drug resistance. Indian J Med Microbiol 2010;28:380–4.

[10] Crivaro V, Bagattini M, Salza MF, Raimondi F, Rossano F, Triassi M, et al. Risk factors for extended-spectrum beta-lactamase-producing Serratia marcescens and Klebsiella pneumoniae acquisition in a neonatal intensive care unit. J Hosp Infect 2007;67:135–41.

[11] Shakil S, Ali SZ, Akram M, Ali SM, Khan AU. Risk factors for extended-spectrum beta-lactamase producing Escherichia coli and Klebsiella pneumoniae acquisition in a neonatal intensive care unit. J Trop Pediatr 2010;56:90–6.

[12] Boo NY, Ng SF, Lim VK. A case–control study of risk factors associated with rectal colonization of extended-spectrum beta-lactamase producing Klebsiella sp. in newborn infants. J Hosp Infect 2005;61:68–74.

[13] Rettedal S, Løhr I, Natas OB, Giske CG, Sundsfjord A, Øymar K. First outbreak of extended-spectrum β-lactamase-producing Klebsiella pneumoniae in a Norwegian neonatal intensive care unit; associated with contaminated breast milk and resolved by strict cohorting. APMIS 2012;120:612–21.

[14] Engel LS. The dilemma of multidrug-resistant Gram-negative bacteria. Am J Med Sci 2010;340:232–7.

[15] Kunz AN, Brook I. Emerging resistant Gram-negative aerobic bacilli in hospital-acquired infections. Chemotherapy 2010;56:492–500.

[16] Brady MT. Health care-associated infections in the neonatal intensive care unit. Am J Infect Control 2005;33:268–75.

[17] Singh N, Patel KM, Leger MM, Short B, Sprague BM, Kalu N, et al. Risk of resistant infections with Enterobacteriaceae in hospitalized neonates. Pediatr Infect Dis J 2002;21: 1029–33.

[18] Boszczowski I, Nicoletti C, Puccini DM, Pinheiro M, Soares RE, Van der Heijden IM, et al. Outbreak of extended spectrum beta-lactamase-producing Klebsiella pneumoniae infection in a neonatal intensive care unit related to onychomycosis in a health care worker. Pediatr Infect Dis J 2005;24:648–50.

[19] Gupta A, Della-Latta P, Todd B, San Gabriel P, Haas J, Wu F, et al. Outbreak of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in a neonatal intensive care unit linked to artificial nails. Infect Control Hosp Epidemiol 2004;25:210–5.