

# Successful elimination of extended-spectrum beta-lactamase (ESBL)-producing nosocomial bacteria at a neonatal intensive care unit

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**Background:** Extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria are highly dangerous to neonates. At our Neonatal Intensive Care Unit (NICU), the presence of these bacteria became so threatening in 2011 that immediate intervention was required.

**Methods:** This study was conducted during a nearly two-year period consisting of three phases: retrospective (9 months), educational (3 months) and prospective (9 months). Based on retrospective data analysis, a complex management plan was devised involving the introduction of the INSURE protocol, changes to the antibiotic regimen, microbiological screening at short intervals, progressive feeding, a safer bathing protocol, staff hand hygiene training and continuous monitoring of the number of newly infected and newly colonized patients. During these intervals, a total of 355 patients were monitored.

**Results:** Both ESBL-producing *Enterobacter cloacae* and *Klebsiella pneumoniae* were found (in both patients and environmental samples). In the prospective period a significant reduction could be seen in the average number of both colonized (26/167 patients;  $P=0.029$ ) and infected (3/167 patients;  $P=0.033$ ) patients compared to data from the retrospective period regarding colonized (72/188 patients) and infected (9/188 patients) patients.

There was a decrease in the average number of patient-days (from 343.72 to 292.44 days per months), though this difference is not significant ( $P=0.058$ ). During the prospective period, indirect hand hygiene compliance showed a significant increase (from the previous 26.02 to 33.6 hand hygiene procedures per patient per hospital day,  $P<0.001$ ).

**Conclusion:** Colonizations and infections were rolled back successfully in a multi-step effort that required an interdisciplinary approach.

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**Key words:** hand hygiene; INSURE protocol; interdisciplinary approach; neonates; polyresistance

## Introduction

The appearance of polyresistant bacteria is a major concern among medical care providers all over the world.<sup>[1-3]</sup> Of these, extended-spectrum beta-lactamase (ESBL)-producing gram-negative bacteria are especially problematic, as they are becoming increasingly resistant.<sup>[4,5]</sup> The group of ESBL-producing bacteria typically includes *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. These bacteria are highly dangerous to neonates, especially low-birthweight preterm infants, and their nosocomial persistence may lead to prolonged hospital stay, higher mortality and growing costs.<sup>[4,6,7]</sup> The increasing presence of nosocomial bacteria is a significant risk factor in neonatal intensive care, which must be dealt with.<sup>[8,9]</sup>

The Neonatal Intensive Care Unit (NICU) of the Department of Pediatrics at the University of Szeged is a 17-bed tertiary care centre, with an annual admission of 210-250 newborns with the most severe perinatal

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conditions from the Southeast region of Hungary (with a population of almost 1.5 million). At this unit, the first ESBL-producing infection was detected in 2002. By 2008, ESBL-producing bacteria became standard nosocomial bacteria, and a total eradication was never achieved. The problem became critical in the second half of 2011, when more than half of the neonates in the ward were colonized, which called for immediate intervention. In this study, we described this complex project in the period January 2011-September 2012, which finally led to successful infection control management at our NICU.

## Methods

As a consequence of the spread of ESBL-producing bacteria, a specific infection-control task force was formed, with representatives from the NICU, the Institute of Clinical Microbiology and the Infection Control Unit. The group met weekly to discuss the situation. A three-step complex management plan was devised in September 2011, which involved retrospective data analysis aimed at identifying risk factors, education of staffs and introduction of new hygienic measures based on the retrospective analysis, and a follow-up phase. We conducted the retrospective study from January 2011 to September 2011 and the prospective study from January 2012 to September 2012. Between the two periods we allowed the staff three months to become accustomed to the new protocols and strategies introduced. Patient-days/month<sup>[10]</sup> were calculated by the electronic patient documentation system (eMedSolution® by T-Systems Hungary Ltd, Budapest), which provides up-to-date data and automatically generates statistical information upon the user's request.

### Retrospective analysis

Data were gathered retrospectively from the January-September 2011 period regarding 1) hand hygiene compliance among healthcare workers and 2) ESBL colonization/infection data among patients treated at the NICU. Hand hygiene compliance was assessed indirectly, based on the recorded use of alcohol-based hand rub (ABHR), from which the average number of hand hygiene procedures could be estimated according to the WHO Guidelines on Hand Hygiene in Health Care.<sup>[11]</sup>

Indirect hand hygiene compliance, which refers to the number of hand hygiene procedures performed in the case of one patient during one day at hospital, was calculated as follows. The quantity of hand hygiene liquid (total millilitres) supplied to

the clinic per month was checked. At the end of the month the quantity of hand hygiene liquid remaining in the dispensers was evaluated (dispensers were marked at the level of remaining product at the end of the month). We managed to count the monthly consumption of ABHR. This amount was divided by 3 mL, because our dispensers provide 3 mL ABHR with each hit. According to studies 3 mL of ABHR is adequate to insure proper hand hygiene<sup>[12,13]</sup> for the user. This calculated number is an indirect measure of the number of hand hygiene procedures performed. This was further divided by patient-days regarding the same month, which is continuously generated by software (eMedSolution®) at our facility. With this formula we are able to calculate the number of hand hygiene procedures performed in the case of one patient during one patient-day at hospital. According to our observations these dispensers were only used by staff, so the number of visitors did not cause a distortion in the results.

Patient files were surveyed for microbiological documentation, in an attempt to determine the types of ESBL-producing bacteria in the ward, and the findings were recorded.

### Preventive measures and prospective analysis

Based on the findings of the retrospective phase, a number of preventive measures were introduced during October-December, 2011.

First of all, in September 2011, the intubation, surfactant therapy and extubation (INSURE) protocol was introduced.<sup>[14]</sup> With INSURE protocol, the mechanical ventilation time can be reduced, which helps reduce ventilation-associated infections.

The antibiotic protocol was also modified. On admission, blood culture and gastric aspirate were collected from each new patient. Ampicillin or a combination of ampicillin and tobramycin was started as primary antibiotic therapy, but the administration of these antibiotics was stopped after 48 hours if the cultures taken on admission proved to be negative and the inflammatory markers were also negative. Similarly, if surface cultures were positive without an elevation of inflammatory markers (i.e. C-reactive protein <10 mg/dL and procalcitonin <10 ng/mL at the age of 24 hours) and the patient did not show clinical signs of infection, positive surface cultures were considered as colonization and the course of antibiotics was discontinued.<sup>[15]</sup> If late onset sepsis was suspected, the choice of antibiotics was changed. Third-generation cephalosporines and a combination of amoxicillin and clavulinate, which are reported to be the strongest inducers of ESBL-production,<sup>[16]</sup> were banned from

the ward. In suspected cases of gram-negative sepsis, meropenem was administered. If there was a predisposition to gram-positive sepsis (e.g. central venous line inserted), vancomycin was also started and the therapy was changed according to the results of the blood culture.

Progressive feeding was started within the first two hours after admission. The neonates received their own mother's breast milk through a gastric tube, if available. If not, premature and neonatal formula were administered. Probiotics containing both bifidobacteria and lactobacilli were also provided.

As a new preventive measure, the neonates were bathed every four days, which is in accordance with the latest guidelines.<sup>[17,18]</sup> Immersion baths were stopped. This was important because the risk of infection by biofilm-forming bacteria (i.e. on the surface of the basin or in the waterlines) could thus be reduced.<sup>[19,20]</sup>

Due to the potential risk of cross infection with ESBL *K. pneumoniae* transferred via the hands of healthcare personnel at the NICU,<sup>[21]</sup> our team has put great emphasize on proper hand hygiene. Hand hygiene training was a central step in the complex intervention. Multiple education sessions were provided for all staff, including video-assisted instruction and hands-on practice. Disinfected hands were also examined under ultraviolet (UV) light to ascertain efficacy. The data collected in the retrospective phase were shared with the staff and information posters were placed at the NICU. The aim was to draw attention to the growing problem of ESBL-producing bacteria in the ward so as to enhance compliance with the infection control and prevention protocol.<sup>[22]</sup>

In order to identify potential reservoirs and risk factors, environmental screening was performed and samples were taken from various surfaces. ESBL-producing bacteria were first detected in these samples in October 2011. Multiple surfaces and areas were colonized, including in the wash basins and taps and a tray used for drying dishes in the nurses' room. Previously, *Pseudomonas aeruginosa* was detected from samples taken from these taps and wash basins. As part of the preventive intervention, samples were taken more frequently (every second month instead of semiannually).

New filters were used on taps and the sinks were regularly dismantled and disinfected, while the staff was instructed that hand washing with soap and water should be done as far as possible at the wash basins outside the hospital rooms. This was important since germs may be emitted as aerosols from the siphon traps into the ambient air during water drainage.<sup>[23]</sup> Additionally, new ABHR dispensers were placed in the ward wherever healthcare procedures are performed.

The quality of cleaning was also evaluated and monitored. In this specific ward the cleaning staff was only allowed to perform cleaning on "non-critical surfaces" (e.g. floor, walls, wash basins), whereas the "critical surfaces" (e.g. therapeutic devices, infant incubator, respiratory devices) were cleaned by the nurses responsible for the care of a particular neonate. The term "critical surface" in this context refers to surfaces which are highly important in terms of the potential spread of healthcare-associated infections. Cleaning of these surfaces was performed multiple times a day according to a more frequent schedule (twice per shift, instead of once per shift).

In order to stop the spread of ESBL-producing bacteria, patients were screened for these on admission, and if the patient presented symptoms of infection at any time during treatment, multiple samples were taken (rectal swab, blood, urine and nasopharyngeal swab). Samples were collected from every patient in contact with an infected patient. Rectal swabs were performed on all neonates not only on admission, but also every other week thereafter. Once colonization or infection was detected, contact precautions were implemented and maintained throughout the hospital stay. Furthermore, colonized and non-colonized babies were nursed separately by individual nurses. Since the ward contained separated boxes for the treatment of the patients, it was easy to implement the separated nursing.

For monitoring the efficacy of our interventions, the number of newly infected and newly colonized patients, monthly costs of antibiotics and monthly mechanical ventilation days were analyzed throughout the prospective period and compared with the data derived from the retrospective interval.

### Microbiological analysis

Identification of isolates was carried out with the conventional biochemical identifications and VITEK GN (bioMérieux, France). Antibiotic susceptibility testing to different antibiotics was performed with a disk diffusion method in line with the CLSI EUCAST recommendation. If necessary, antibiotic minimum inhibitory concentration (MIC) value was determined with gradient MIC test strips (Liofilchem, Roseto, Italy), and the results were evaluated according to the EUCAST guidelines.<sup>[24]</sup> The putative production of an ESBL was detected with the ESBL Detection Set (MAST Diagnostica, Reinfeld, Germany) or the modified double-disk synergy test using ceftazidime (30 µg), cefotaxime (30 µg), aztreonam (30 µg) and cefepime (30 µg) disks opposite an amoxicillin (20 mg)/clavulanic acid (10 mg) disk. The *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub> genes were detected and characterised as described

previously (ESBL-PCR).<sup>[25]</sup> Genetic relationships between *Klebsiella pneumoniae* and *Enterobacter cloacae* isolates were investigated with the pulsed field electrophoresis method (PFGE) using *Xba*I restriction endonuclease according to the standardized PulseNet protocol.<sup>[26]</sup> Results were interpreted and pulsotypes were assigned in line with the criteria set by Tenover et al.<sup>[27]</sup> The selected *K. pneumoniae* blood isolate was subjected to multilocus sequence typing (MLST) according to Diancourt et al.<sup>[28]</sup>

### Statistical analyses

The Mann-Whitney *U*-test, Wilcoxon-test, and Student's *t*-test were used, as appropriate. Level of significance was set at  $P < 0.05$ . Statistical analyses were performed with SPSS 19 (IBM Corp., Armonk, NY, USA).

### Results

Compared with the retrospective period, during the prospective period the average number of patient-days decreased from 343.72 days per month to 292.44 days per month, though this difference is not significant ( $P = 0.058$ ). During the prospective period, a significant reduction was observed both in the number of colonized (from 72/188 to 26/167;  $P = 0.029$ ) and infected patients (from 9/188 to 3/167;  $P = 0.033$ ) when compared to the retrospective examination interval. It is worth mentioning that in the retrospective period five infected patients died, while no deaths occurred after the introduction of the new measures.

The number of invasive mechanical ventilation days per patient care days was also decreased significantly,

almost by 50% (Table). There was no statistically significant difference between the two examination phases in terms of the cost of antibiotic consumption related to patient-days (from 7.5 euros/patient-day to 6.3 euros/patient-day,  $P = 0.519$ ).

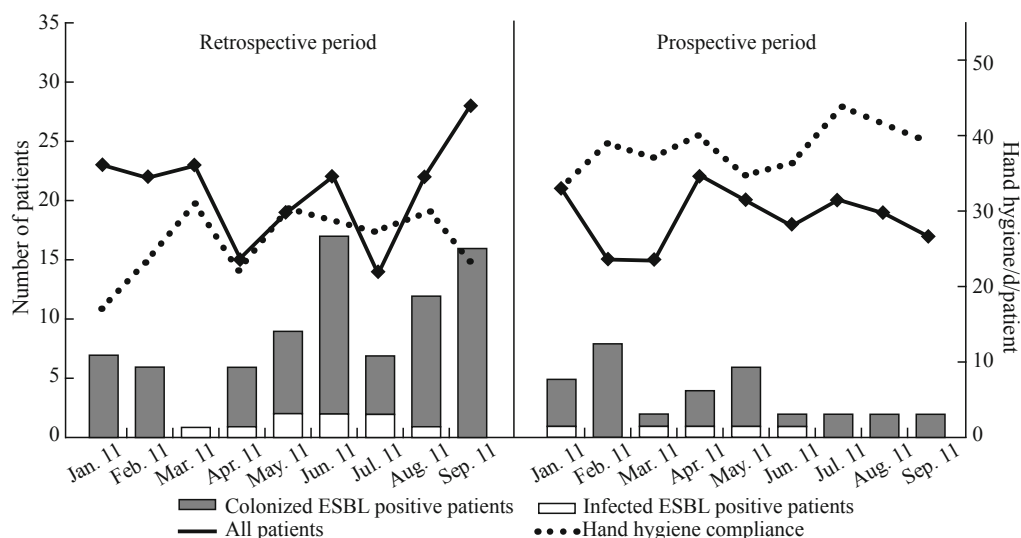
Regarding the samples taken from the ESBL-positive patients, during the whole interval, 26 out of 29 *K. pneumoniae* isolates exhibited pulsotype Z, and the remaining three isolated were of the KP083, KP085 and L pulsotypes. The pulsotype Z isolate belonged to sequence type 525 and harboured the *bla*<sub>CTX-M-15</sub> ESBL gene. As for the 25 *E. cloacae* isolates studied, 23 belonged to *bla*<sub>SHV</sub>-bearing pulsotype EbC052, one to EbC054 and another to EbC038.

Totally 170 environmental swab samples were taken

**Table.** Descriptive statistics of the study parameters from the two examined periods

Variables	2011 January-September retrospective period	2012 January-September prospective period
Patient day	324.50 (306.00-403.50)	296.00 (175.50-376.50)
Admitted patients/mon	22 (14-28)	19 (15-22)
ESBL colonized patients	7 (1-15)	2 (0-8)*
ESBL nosocomial infected patients	2 (0-4)	0 (0-1)*
ABHR consumption/L	26.50 (19.5-34.5)	32.50 (23.0-46.4)*
Monthly mechanical ventilation day/ventilated patients	9.77 (5.88-18.11)	5.00 (3.24-8.88)†
Performed hand hygiene/patient/day	27.39 (17.22-31.08)	39.17 (33.28-44.07)‡

Values are given as median (minimum-maximum). \*:  $P < 0.05$ ; †:  $P < 0.01$ ; ‡:  $P < 0.001$ . ESBL: extended-spectrum beta-lactamase; ABHR: alcohol-based hand rub.



**Fig.** Comparison of the retrospective and prospective periods in terms of colonization, infection and hand hygiene compliance "hand hygiene/d/patient" refers to the number of hand hygiene procedures performed in the case of one patient during one day at hospital.



during the intervention period (October to December 2011). These samples were collected from 107 critical and 63 non-critical surfaces. Twenty-five out of 107 critical and 14 out of 63 non-critical surface samples were culture positive, respectively, thus highlighting inadequately cleaned areas. Of these samples, a few were taken from a wash basin, three taps, a common warming bath for feeding bottles and the dish tray in the nurses' room; all of these contained ESBL *Enterobacter cloacae*, belonging to pulsotype EbC052. In February 2012, we also took samples from healthcare workers' stool in search of ESBL-producing bacteria and further potential sources of infection because these caregivers are in direct close contact with the infants. Two samples from 32 healthcare workers showed ESBL *E. coli* positivity; however, no infants showed ESBL *E. coli* positivity at the ward.

In a comparison of the two periods under examination, a significant increase can be seen during the prospective period regarding the consumption of ABHR solutions ( $P=0.03$ ). In the first phase, this represented an average of 77.90 L ABHR per 1000 patient-days, while in the second interval, this figure increased to 114.96 L per 1000 patient-days. Compared with the baseline data at the beginning of the retrospective period under examination (26.18 ESBL-positive patients per 1000 patient-days), a significant reduction can be seen in the incidence of ESBL-positive patients by the end of the prospective phase (11.01 positive patients per 1000 patient-days,  $P=0.02$ ). Indirect hand hygiene compliance showed a significant increase in the prospective examination period compared to the retrospective examination period ( $P<0.001$ ) (Fig.). During the retrospective period, 26.02 hand hygiene procedures were performed on average per patient per hospital day, and this increased to 33.6 during the prospective period. As a result of the hand hygiene education and with the useful aid of a UV lamp for supervision, the efficacy of hand cleaning among healthcare workers also improved significantly. In the retrospective period, when hand hygiene practice was examined, the nail beds and dorsal surfaces of the thumbs were usually missed (perfect results were only achieved in 14% of the cases). During a three-week period, staff was supplied with a UV lamp for detecting fluorescent ABHR to provide them with an opportunity to practice and evaluate their own hand hygiene technique. During the UV lamp-supported training, perfect hand hygiene practice increased to 77%.

## Discussion

Infection control has a remarkable historical connection with the pediatric population. Ignác Semmelweis

already found a link between hand hygiene and perinatal infection rates in the nineteenth century.<sup>[29]</sup>

The aim of our intervention was to roll back colonization and infections caused by ESBL-producing bacteria at our NICU, and as our results suggest, we have managed to reach that aim.

Most importantly, there was a sharp decrease in the number of patients colonized and infected with ESBL-producing bacteria. Invasive mechanical ventilation days were successfully reduced almost by half. This result is mostly due to the introduction of INSURE therapy.<sup>[30,31]</sup> However, INSURE not only caused a decrease, but also reduced the chance of ventilator-associated infections and so contributed to the drop in late bacterial colonizations and the occurrence of late onset sepsis and thus may have contributed to the fall in late invasive ventilatory support demand.<sup>[32]</sup>

ESBL-producing Gram-negative bacteria can survive on environmental surfaces, preferably in moist sites, for weeks; environmental decontamination is therefore a highly important issue in intensive care units.<sup>[33]</sup> In accordance to other studies,<sup>[34-36]</sup> we also detected ESBL-producing bacteria on moist surfaces and places. After an evaluation of the results and an identification of possible sources, the use of wash basins was minimized and the dish tray was removed permanently from the nurses' room. Also, the local specific warming method (i.e. that all feeding bottles were warmed in a common warming bath) was immediately banned from the ward and the bottles were warmed individually from that point on. As a further preventive measure, the water in these new individual warming devices was also changed several times a day, and devices were cleaned with disinfectant solutions after use and stored dry. This intervention caused a remarkable reduction in the number of new ESBL-positive patients.

In the study carried out by Rong et al,<sup>[21]</sup> the potential sources of infection at the NICU were the gastric tubes, the incubators and the healthcare personnel. Although none of the mentioned surfaces or the care providing staff were justified as real sources of infection, with the multistep intervention we managed to improve these fields too. The quality of cleaning critical surfaces near the patients improved, as none of these surfaces have produced positive samples after the introduction of the new cleaning regimen.

We also carried out faecal sampling from healthcare workers' stool in search of ESBL-producing bacteria. Although two samples from 32 healthcare workers exhibited ESBL *E. coli* positivity, no infants showed ESBL *E. coli* positivity at the ward. The staff members, who were colonized with ESBL *E. coli*, therefore,

cannot be regarded as potential sources of the infection under examination.

Finally, as a result of the hygienic interventions, namely the examination of hands under UV light and small-group training sessions for clinicians and staff, including instruction on correct hand hygiene procedure, indirect hand hygiene compliance showed a significant increase. Our results are in accordance with Zahar et al,<sup>[37]</sup> who also found a significant rise in ABHR use in the period following interventions.

In conclusion, rolling back ESBL-producing bacteria at our NICU was successful. We attribute this success mainly to the multidisciplinary approach, the continuous feedback and monitoring, and the high compliance of the staff. Although the staff of a NICU is in closer contact with neonates, compared to a ward with older patients, colonization of health care workers did not play any role in the nosocomial persistence of ESBL-producing bacteria.

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**Competing interest:** The authors report no declarations of interest.

**Contributors:** All authors contributed to the manuscript conception, design, drafting, revision and appraisal.

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### Erratum: author's name correction

In the article by Sheikh SI, et al, "Racial differences in pet ownership in families of children with asthma" in *World Journal of Pediatrics* 2016;12(3):343-346 (doi: 10.1007/s12519-016-0027-9), the last author's name was incorrectly listed as "Don Hayes". His name should have been "Don Hayes Jr."

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