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Occurrence and spread of SHV extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* isolates in Curaçao

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Sir,

In recent years, the growing incidence of extended-spectrum β -lactamases (ESBLs), in particular produced by *Klebsiella pneumoniae* isolates in nosocomial infections, has also become evident in Curaçao (Netherlands Antilles). Because of the island's location, the occurrence of ESBLs may be a consequence of local emergence or import from other countries.¹ To identify a possible source for the increase in these multidrug-resistant isolates, 65 non-duplicate clinical isolates of ESBL-producing *K. pneumoniae* were investigated having been collected in the St Elisabeth Hospital from November 1999 to June 2002.

Identification and susceptibility testing were carried out using an automated system (Vitek, bioMérieux Vitek Inc., Hazelwood, USA). When MICs indicated the potential presence of an ESBL, a double-disc synergy test (DDST) was used to confirm ESBL production.² Ribotyping using *EcoRI* was carried out with an automated riboprinter (Qualicon Europe Ltd., Warwick, UK) as described previously.³ Fourteen isolates collected during 2001 from an outbreak in the neonatal intensive care unit (NICU) were considered separately, because of the isolated character of this event. Twelve isolates were collected from November 1999 to December 2000 (group A). Thirteen isolates were collected outside the NICU, during 2001 (group B). Twenty-six isolates were collected in the first 6 months of 2002 (group C). There was a significant increase in the isolation of ESBL-producing *K. pneumoniae* isolates in Curaçao, from less than one per month in 1999/2000 to approximately five per month in the first half of 2002, which was only partly explained by better screening methods.

Twenty-two distinct ribotype patterns were identified (Figure 1) and were compared to an international database containing more than 1800 ribotype patterns that was constructed during the framework of the Genetic Epidemiology Network for Europe (GENE) project (www.ewi.med.uu.nl/gene). Ten of the 22 patterns, found in 42 Curaçao isolates, were identical to patterns in the GENE database and most were found in different countries. Interestingly, ribotype 77 was the most represented type (26 isolates) in Curaçao and was also the ribotype with the largest number of isolates (98 isolates) of the 10 matching patterns from the GENE database. This ribotype was mainly found in the Americas, but also in Europe. Other geographically widespread ribotypes, found both in the Americas

and in Europe, were ribotypes 122 (44 isolates in the GENE database), 438 (38 isolates) and 38 (22 isolates). These results indicate that most Curaçao *klebsiellae* in our study (42 out of 65 isolates) do not represent endemic strains but are exchanged between the island and other parts of the world, and also many of these imported strains have a wide geographical distribution.

To identify the β -lactamase profiles in these *K. pneumoniae* isolates, isoelectric focusing (IEF) was carried out. Most of the non-NICU isolates demonstrated a variety of IEF patterns, and 38 of the 51 non-NICU (75%) isolates showed a $pI \geq 8.3$ suggestive of a CTX-M-type or AmpC β -lactamase. To detect possible CTX-M β -lactamases, isolates were screened with three sets of primers.⁴ All isolates were negative. The presence of a DHA-type AmpC enzyme was confirmed by PCR amplification in eight isolates (12%).⁵ To assess whether *bla*_{SHV}-type β -lactamases were present in our isolates, PCR amplification with the primers SHV-*Nhe*-F (5'-GAGCGAAAGATCCACTTCG-3') and SHV-*Nhe*-R (5'-GTATCCCAGAT-AAATCA-3') was carried out to detect a 525 bp *bla*_{SHV} gene-specific fragment (GenBank accession no. AF124984[SHV1], bp 262–281 and bp 786–767). The majority of SHV-type ESBLs can be detected by restriction analysis of the amplicon by *NheI*.⁶ Sixty-three isolates showed an amplification product in the SHV-*NheI*-PCR. Fifty-four isolates showed two *NheI*-digestion products and were considered SHV-ESBL positive. The 11 isolates that yielded no digestion products had a positive DDST, but their ESBL phenotype was probably the result of another type of β -lactamase; these isolates were collected early in the study (group A). Sixty-two of 65 isolates showed transferable ampicillin resistance; in 24 isolates, this was associated with transfer of genes encoding SHV ESBLs. Almost all of the isolates from the NICU outbreak of 2001 yielded SHV transconjugants. However, for the non-NICU isolates, the conjugation rate for SHV was low (15%). This may be explained by poor mobilization of the SHV carrying plasmid or competition with other resistance plasmids.

Our data indicate that there is an ongoing outbreak of ESBL-producing *K. pneumoniae* in Curaçao. Most of the ESBL-producing isolates (83%) appear to carry plasmid-encoded SHV-type β -lactamases and this plasmid is not easily transferable from the majority of isolates. Isoelectric focusing indicated that most of the isolates harbour a diversity of β -lactamase genes, with an AmpC-type present in 12%. There is a prevalent clone, whose ribotype pattern indicated that it is probably imported from the Americas or Europe.

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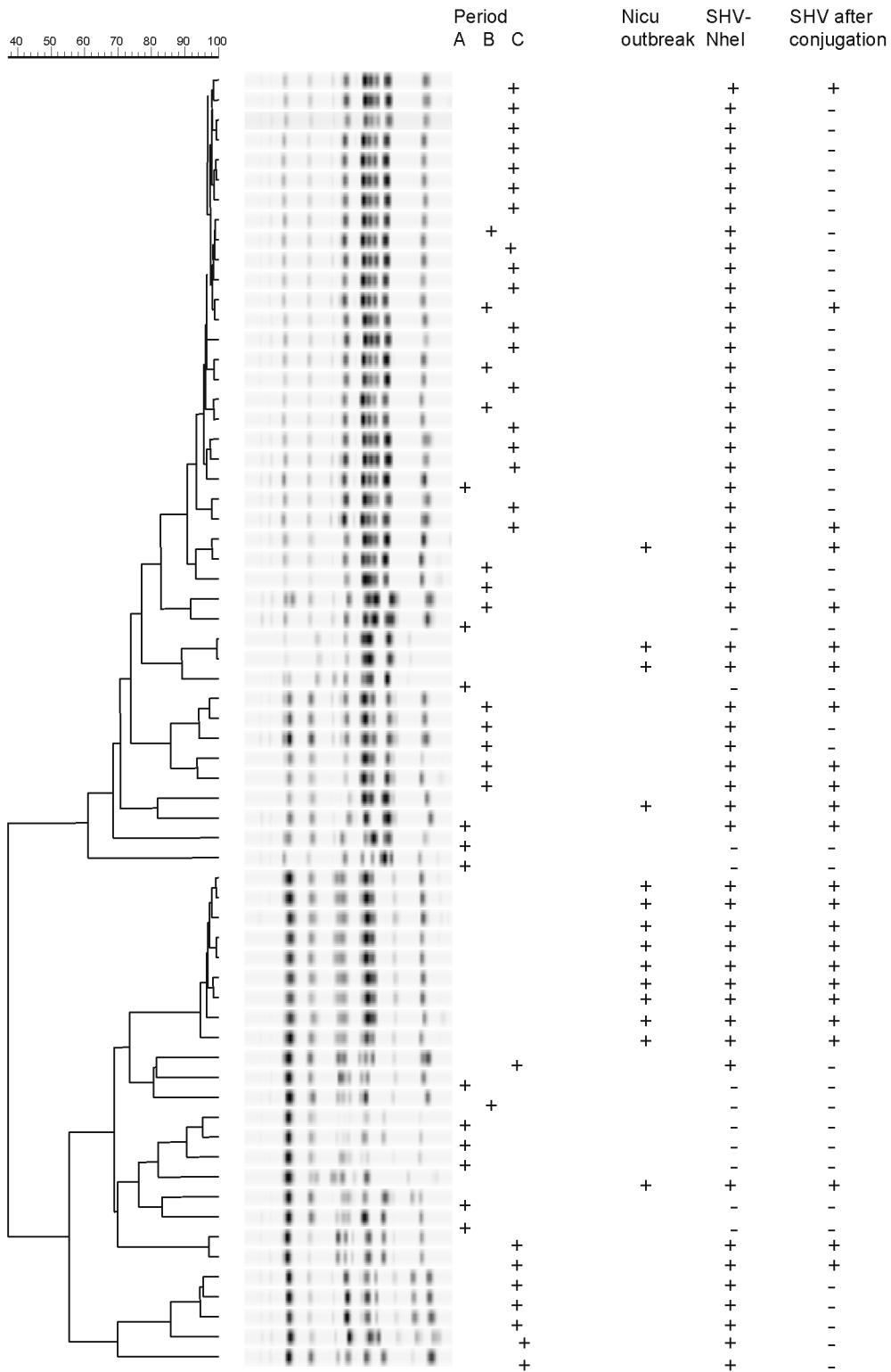


Figure 1. Results from ribotyping, SHV-RFLP (*NheI* PCR) and conjugation experiments for 65 ESBL-producing *K. pneumoniae* collected in the St Elisabeth Hospital in Curaçao. A, 1999/2000; B, 2001; C, 2002.

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Absence of vancomycin-resistant enterococci (VRE) in companion dogs in the conurbation of Parkstad Limburg, The Netherlands

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Keywords: veterinary, prevalence, avoparcin, glycopeptide resistance

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Sir,

As the epidemic occurrence of vancomycin-resistant enterococci (VRE) has been recently noted in patients in The Netherlands, vigilance for this kind of antibiotic resistance is clearly indicated.¹ VRE have already been demonstrated in poultry farmers in our region in The Netherlands.² The presence of VRE in 16% of companion dogs has been noted in a Dutch urban veterinary practice in the city of Rotterdam.³ How these dogs became colonized by VRE is still unknown. Culture of various dog foods revealed no VRE (Dr H. Ph. Endtz, Erasmus MC, University Medical Center, Rotterdam, personal communication). The occurrence of VRE in companion dogs can have implications for transfer of these VRE (frequently *Enterococcus faecium*) to humans.⁴ Therefore, in 2002, we initiated a study into the occurrence of VRE in companion dogs in a comparable setting in the conurbation of Parkstad (Parkcity) Limburg, The Netherlands.

The test procedure was as follows: after informed consent of the dogs' owners, a rectal swab was taken from 100 dogs, visiting the animal clinic for various clinical reasons. The swab was placed in a

non-selective enrichment tryptone broth, incubated aerobically for 48 h at 37°C and subcultured on an agar blood plate with selective antibiotics (ceftazidime 8 mg/L and gentamicin 10 mg/L) to prevent the overgrowth by other bowel bacteria and favouring the overgrowth of enterococci. On the primary inoculation streak, a 30 μ g vancomycin disc was placed as an initial screening. Suspected colonies were further processed for identification and susceptibility testing according to standard procedures.

No VRE were detected in any of these 100 companion urban dogs contrary to our initial expectation in view of the marked prevalence in urban dogs from the same sort of city environment 6 years earlier.³ If one or more dogs had been detected as a carrier of VRE, it would have been of interest to investigate the eating habits of the dogs implicated. With no VRE found, this topic was not pursued further. The current absence of VRE in these companion animals can possibly be explained by the fact that nearly 5 years had passed since the introduction of the ban on avoparcin for animal feeding by order of the European Commission in 1997. This action has already caused a significant downward trend in the prevalence rates of VRE in other previously, frequently identified groups of VRE-colonized farm animals.⁵ This communication may contrast with the usual pessimistic reports of increasing prevalences of bacterial resistance.

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