Journal of Hospital Infection (2000) **45**: 135–144
doi:10.1053/jhin.2000.0728, available online at http://www.idealibrary.com on IDEAL®

Nosocomial outbreak of ampicillin resistant *Enterococcus faecium*: Risk factors for infection and fatal outcome

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Summary: A nosocomial outbreak caused by ampicillin resistant *Enterococcus faecium* (ARE) was detected at a Norwegian university hospital in January 1995. Prior to this outbreak, ARE were not common in this hospital or other hospitals in Norway. During 1995 and 1996, a total of 149 cases with clinical ARE infection were detected prospectively. A case control study was performed by allocating controls matched for gender, age and ward of admission. Altogether, 123 case control pairs with mean age 70.1 years were included. Isolates from 89 (72.4%) of the cases were identical or related to the defined outbreak strain as determined by pulsed-field gel electrophoresis (PFGE). In 75 of the patients (60.9%), ARE caused urinary tract infection, five (4.1%) had bacteraemia, 33 (26.8%) had wound infection and 10 (8.1%) had other infections. In a logistic regression model for 1:1 matched samples, the following factors were identified as significant risk factors for ARE infection: underlying neurological disease (OR = 33.5), prescription of antimicrobial agents for more than 10 days (OR = 8.99), prescription of cephalosporins (OR = 4.69), underlying gastrointestinal disease (OR = 3.36) and length of hospital stay per day (OR = 1.04). The intrahospital death rate for the cases was 18.7% compared with 8.9% for the controls, corresponding to an excess mortality attributable to ARE infection of 9.8%. A history of carbapenem prescription was the only independent factor contributing to death (OR = 5.64) when comparing ARE patients dying in hospital to those surviving.

Keywords: Enterococcus faecium; ampicillin resistance; cephalosporins; disease outbreaks.

Introduction

The emergence of enterococci as significant pathogens is worrying because these organisms are inherently resistant to a number of antimicrobial agents, including cephalosporins and aminoglycosides. Penicillins and glycopeptides are only bacteriostatic. The limited choice of efficient therapy in serious enterococcal infections is complicated by the emergence of ampicillin resistance, high-level aminoglycoside resistance and glycopeptide resistance.[1] In recent years, enterococci have emerged as a significant cause of nosocomial infections,[2,3] accounting for about 8% of hospital-acquired infections in the USA.[1] Urinary tract infections are the most common, but enterococci are also frequently isolated from abdominal and surgical wound infections, and are important causes of bacteraemia and endocarditis.[4–7] The majority of clinical enterococcal isolates belong to the *Enterococcus faecalis* species, but there are increasing number of reports on infections caused by *Enterococcus faecium*.[1]

There have been few reports on ampicillin resistant enterococci (ARE), vancomycin resistant enterococci (VRE) or high-level aminoglycoside

Received 17 November 1999; manuscript accepted 12 January 2000.
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0195–6701/00/020135+10 $35.00

resistant enterococci from Scandinavia.[8–14] Only very few infections caused by VRE have been diagnosed in Norway[8,15,16] and faecal carriers of VRE are rare.[13,17] The prevalence of ARE infection has also been low in this country until recently[14,16], although faecal carriers of ampicillin resistant enterococci have been found among patients in selected hospital departments.[13] We discovered a large nosocomial outbreak caused by ARE in our hospital, starting in 1995.[10,14,18] More than 300 patients with clinical infections have since been identified. We have also shown that a significant proportion of patients in some wards become faecal carriers of the outbreak strain.[19]

Little is known about the spread of ARE in hospitals, and only small studies of risk factors for infection have been published.[5,20–22] Exposure to antimicrobial agents, prolonged hospital stay and insufficient infection control practices are among the suspected risk factors. A little more is known about the spread and risk factors for VRE, where use of third generation cephalosporins and vancomycin have been found to represent a significant risk for the patients.[23] A large proportion of clinical VRE isolates are also resistant to ampicillin.[24] It is therefore difficult to assess risk factors for ARE infection in epidemiological settings where VRE are established. In the present epidemiological study of a large ARE outbreak at Haukeland University Hospital, we have been able to assess risk factors for ARE infection in a setting where VRE infections are rare.

Materials and methods

Setting

Haukeland University Hospital has 1,100 beds and serves 950,000 people as a referral hospital and 350,000 people as an emergency hospital. The Department of Microbiology and Immunology at Haukeland University Hospital serves the only two hospitals in the city of Bergen and also the three other emergency hospitals located in Hordaland County. It is also the main bacteriological service for outpatients in Hordaland County (431,000 inhabitants). The number of specimens examined each year exceeds 80,000, of which about 50,000 are from outpatients.

Before 1995, only two clinical ARE isolates had been observed. During 1995 and 1996, ARE isolates were grown from clinical specimens of 149 patients of which only four isolates were resistant to vancomycin.[14] There are also two collaborating microbiological laboratories in the nearest two counties serving the rest of the hospitals and outpatients in the region. With the exception of one clinical ARE and VRE case, these two laboratories did not report any occurrence of ARE or VRE during 1995 and 1996.

Microbiological methods

All enterococcal isolates from clinical specimens detected by the microbiological routine laboratory were identified at species level by standard biochemical methods.[25] The susceptibility of the isolates to different antimicrobial agents (ampicillin, netilmicin, gentamicin, vancomycin and teicoplanin) was examined by an agar diffusion method[26] using paper disks and PDM Antibiotic Sensitivity Medium (AB Biodisk, Solna, Sweden). Urine isolates were also tested against ciprofloxacin, co-trimoxazole, and nitrofurantoin using the same method. The susceptibilities were categorized as sensitive, intermediate or resistant, according to the recommendations of the Norwegian Working Group on Antibiotics.[27] All isolates classified as resistant to aminoglycosides were examined for high-level gentamicin resistance by the appropriate E-test (AB Biodisk). The minimum inhibitory concentrations (MICs) of ampicillin, vancomycin and teicoplanin were determined for the majority of ARE isolates during the outbreak by E-test (AB Biodisk). Ampicillin resistant *E. faecium* isolates from 102 patients were available for PFGE which was performed on *Sma*I (Promega Corp, Madison, WI, USA) digested genomic DNA as previously described[28] using the Rothapor type V electrophoresis unit (Biometra GmbH, Germany). DNA digests were loaded onto 1% agarose gels and run with 2–15 s pulses at 180 V and 22°C for 20 h. The resulting patterns were interpreted as described.[29] Isolates with different PFGE types were verified as *E. faecium* by a polymerase chain reaction (PCR).[30]

The outbreak strain

The first ampicillin resistant *E. faecium* isolated at the hospital, later shown to represent the dominant PFGE-type, was defined as the outbreak strain. Isolates from 89 patients (87.3%) of 102 available for PFGE were identical or related to this outbreak

strain. All but four isolates were fully susceptible to glycopeptides, but resistant to all other antimicrobial agents tested except nitrofurantoin. The minimum inhibitory concentration for ampicillin was typically 64 mg/L; no strains were high-level gentamicin resistant. The four vancomycin resistant strains were vanB-type with MIC range 8–12 mg/L.[14]

The outbreak

The ARE index case was discovered in the hospital in January 1995.[14] Up until October 15, 1999, 330 patients with ARE infection were identified, of which 284 were patients admitted to Haukeland University Hospital or its outpatient services, and the remaining 46 were admitted to other hospitals. The incidence at Haukeland University Hospital peaked at 2.08 per 1,000 patients in the second quarter of 1996 and declined to 0.96 per 1,000 patients in the fourth quarter of 1998. All the ARE infections were classified as nosocomial and one third of the patients had been hospitalized in one of three wards serving infectious diseases and haematology prior to their ARE infection.

Inclusion criteria and matching

All patients at Haukeland University Hospital with clinical infections caused by ARE during 1995 and 1996 were recorded prospectively and a total of 149 was identified. By means of the hospital computer system, one control patient was matched with each ARE patient by the following criteria: same age (± 3 years), same gender and admission to the same ward. The patient with admission time closest to the case was chosen.

Collection of clinical data

Two skilled infectious disease specialists reviewed the medical records of both cases and controls. The following data were recorded: age, gender, admission time, length of hospital stay, underlying diseases, outcome, use of medical devices (indwelling urinary catheters, central intravenous lines), surgical procedures, antibiotics prescribed during the hospital stay, ongoing antibiotic treatment at the time of admission and the number of days on antibiotic treatment. As ARE infection was the criterion defining entry into the study, potential risk factors such as medical procedures and treatment with antimicrobial agents were recorded only up to the date of the first specimen growing ARE. The underlying diseases were recorded from the discharge diagnosis according to the main groups in ICD 9. The severity of underlying diseases was classified as non-fatal, ultimately fatal, or rapidly fatal, as described previously.[31–33] The ARE infection was classified according to the CDC definitions for nosocomial infection.[34,35]

Statistical methods

The continuous variables for the case and control groups were compared using Student's *t*-test for paired samples and the dichotomous data were examined by McNemar's chi-square test for paired data (two- sided). We applied the McNemar's test to identify possible risk factors for ARE infection. Number of days in hospital was dichotomized as seven days or more in hospital, days on antimicrobial treatment as 10 days or more on antimicrobial agents, and number of different agents prescribed as two or more. Odds ratios (OR) with exact 95% confidence intervals (CI) were estimated using the formula described by Breslow and Day.[36]

A backward conditional logistic regression analysis[36] was performed to assess risk factors for ARE infection.[37] To obtain reasonable stable estimates and convergence in the estimation procedure, variables without observed case-control pairs which were both exposed, and variables with at most 10 discordant pairs, were excluded from the starting model. The remaining variables are specified in the results section.

A univariate analysis of the risk factors for intrahospital death for patients infected with ARE was performed by estimating odds ratios for all the dichotomous variables for the ARE patients dying in the hospital compared with the ARE patients surviving. The significance was tested by the Fisher exact test. All the variables were thereafter entered in a forward stepwise logistic regression analysis (SPSS version 9.0, SPSS Inc., Chicago, IL, USA) with intrahospital death as the dependent variable.

Results

Comparison of included and not included cases

The distribution of all 149 ARE infected patients and of the 123 cases included in the study is shown in Figure 1. We succeeded in finding matching controls and complete data for 123 patient pairs, 52 male and 71 female (Table I). The mean age for cases and

controls was 70.1 years (range 14.5–95.6) and the mean age difference between the cases and controls was 0.35 years.

Of the remaining 26 patients with ARE infections in the study period, we did not succeed in matching one patient, nine patients were outpatients and 16 patients were hospitalized at a nearby emergency hospital from which we could not include appropriate controls. The 26 (13 males, 13 females) who of the 149 ARE patients were not included in this study were compared to the 123 cases included as far as data were available. There were no significant differences in mean age (71.7 vs. 70.3), days in hospital (34.1 vs. 45.1), number of antibiotics prescribed (2.5 vs. 2.8) or number of days on antimicrobial treatment (17.9 vs. 21.7).

Further analyses were therefore performed on the 123 case control pairs included. Seventy-five of the ARE cases (60.9%) had urinary tract infection, five (4.1%) bacteraemia, 33 (26.8%) wound infection and 10 (8.1%) other infections. ARE isolates from 89 (72.4%) of the patients were classified as identical or closely related to the outbreak strain, 13 (10.6%) were not related and 21 isolates were not available for PFGE.[18]

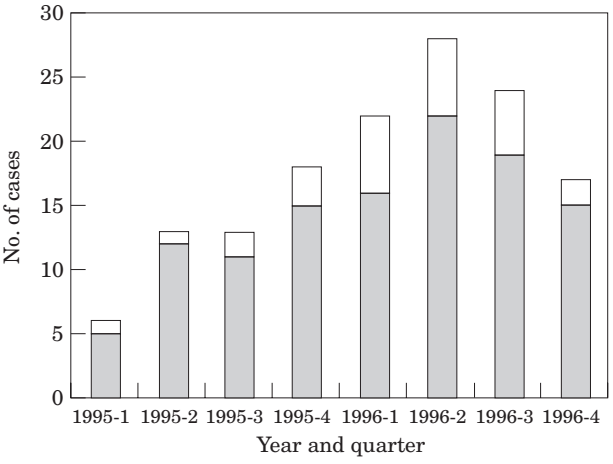

Figure 1 Epidemic curve of all patients infected by ampicillin resistant *Enterococcus faecium* (ARE) ($N=149$) at Haukeland University Hospital during 1995 and 1996, and those included in the case-control study ($N=123$). ▩ 123 ARE cases included in the study; □ other ARE cases.

Unadjusted analyses of possible risk factors for ARE infection

The 123 included ARE cases had significantly longer hospital stays than the controls (45.1 vs. 12.1 days) (Table I). There was no statistically significant difference in surgery performed in the cases and the controls (23.6% vs. 17.1%) (Table II). Indwelling urinary catheters and central venous lines were used significantly more often in ARE infected as compared to control patients (45.5% vs. 26.8% and 29.3% vs. 8.9%, respectively). The cases had been, prior to their ARE diagnosis, treated with antimicrobial agents significantly longer than the control patients (21.7 vs. 5.2 days) (Table I). The mean number of antimicrobial agents prescribed was significantly higher for the cases compared with the controls (2.8 vs. 0.9) (Table II).

Table II shows that several of the variables examined were possible risk factors for ARE infection as they were statistically significant in univariate analysis. The highest OR was found for prescription of antimicrobial agents for 10 days or more (OR = 34.5). Significantly increased risk was also found for prescription of more than two

Table I *Epidemiological data for 123 patients infected with ampicillin resistant* Enterococcus faecium

Factor	ARE patients $N=123$	Control patients $N=123$	*P*-value (Paired *t*-test)
Age, mean (in years)	70.3	69.9	0.865
Female to male ratio	71/52	71/52	–
Days in hospital	45.1	12.1	<0.001
No. of antimicrobial agents prescribed	2.8	0.9	<0.001
Days on antimicrobial treatment	21.7	5.2	<0.001

Enterococcus faecium (ARE) compared to individual control patients matched by age, gender and ward.

Table II *Univariate analysis of possible risk factors for infection with ampicillin resistant* Enterococcus faecium (*ARE*) *in 123 cases compared to 123 matched patient controls without infection*

Factor	ARE cases $N=123$	Patient controls $N=123$	Odds ratio (OR)	95% confidence interval	McNemar χ^2-test P-value
Days in hospital >7	106	58	10.60	4.27–33.98	<0.0001
Intrahospital death	23	11	3.00	1.14–9.23	0.0143
Underlying diseases					
Cardiovascular	16	39	0.26	0.10–0.57	0.0002
Gastrointestinal	21	14	1.78	0.74–4.56	0.1615
Endocrinological	11	9	1.25	0.44–3.64	0.6374
Haematological cancer	20	9	6.50	1.47–59.33	0.0045
Solitary cancer	21	20	1.08	0.46–2.60	0.8415
Pulmonary	2	4	0.50	0.05–3.49	0.4142
Infections other than ARE	48	30	2.06	1.12–3.92	0.0126
Neurological	13	4	4.00	1.08–22.09	0.0201
Bone and joints	8	7	1.25	0.27–6.30	0.7389
Urogenital	12	12	1.00	0.33–3.06	1.0000
Trauma	3	6	0.25	0.01–2.53	0.1797
Immunological	9	5	1.80	0.54–6.84	0.2850
Other	4	9	0.44	0.10–1.59	0.1655
Prescription of antimicrobial agents					
Treatment >10 days	83	16	34.50	9.20–290.57	<0.0001
Number of agents >2	61	11	17.67	5.73–88.39	<0.0001
Penicillins	60	40	2.11	1.18–3.93	0.0075
Cephalosporins	66	21	10.00	4.01–23.13	<0.0001
Imipenem	22	3	7.33	2.20–38.27	0.0001
Fluoroquinolones	37	11	5.33	2.20–15.60	<0.0001
Aminoglycosides	39	10	4.63	2.12–11.50	<0.0001
Metronidazole	24	8	3.29	1.36–9.07	0.0035
Glycopeptides	10	1	10.00	1.42–433.98	0.0067
Co-trimoxazole	14	3	4.67	1.30–25.33	0.0067
Clindamycin	12	1	12.00	1.78–512.97	0.0023
Penicillins and aminoglycosides	26	8	3.57	1.50–9.78	0.0015
Medical procedures					
Surgery	29	21	1.67	0.78–3.74	0.1573
Urinary catheterization	56	33	2.64	1.40–5.29	0.0013
Central venous line	36	11	4.13	1.87–10.34	0.0001

antimicrobial agents (OR = 17.7) and for hospital stay longer than seven days (OR = 10.6). Some underlying diseases (such as haematological malignancy), use of urinary catheter, use of central intravenous line and intrahospital death were also statistically significant risk factors in univariate analysis. Prescription of one or more of several broad spectrum antimicrobial agents was also a risk factor. For cephalosporins, we did not find any significant differences between the different generations of agents (data not shown). For penicillins, we found that isoxazolylpenicillins, in contrast to the other penicillins, represented a significantly higher risk (OR = 2.8). The only factor that seemed to be protective was underlying cardiovascular disease (OR = 0.26).

Multivariate analysis of possible risk factors for ARE infection

The statistically significant risk factors are summarized in Table III. Underlying neurological disease was a risk factor for ARE infection (OR = 33.5). However, only 13 patients and 4 controls had underlying neurological diseases. The other independent risk factors were more than 10 days' antimicrobial treatment (OR = 8.99), prescription of cephalosporins (OR = 4.69), underlying gastrointestinal disease (OR = 3.36) and length of hospital stay per day (OR = 1.04). This means that a hospital stay of one month represents an increased risk of $1.04^{30} = 3.24$. The following variables included in the analysis did not have any significant impact as risk factors and were therefore omitted from the final

model: cardiovascular diseases, endocrinological diseases, haematological malignancies, solitary cancer, infections other than ARE infection, urogenital diseases and diseases other than those classified, number of antimicrobial agents prescribed, prescription of fluoroquinolones, metronidazole, glycopeptides, penicillins alone or penicillins in combination with an aminoglycoside, surgery performed, urinary catheter, central venous catheter or intrahospital death.

The analysis of subsets of data including only patients with an ARE isolate belonging to the outbreak clone-complex or only patients with urinary tract infection and their controls matched respectively did not reveal any new significant risk factors (data not shown).

Outcome

The intrahospital death rate for the cases was 18.7% compared to 8.9% for the controls (Table II), corresponding to 9.8% excess mortality attributable to ARE infection. There were no significant differences between the cases and the controls regarding the severity of underlying diseases (Table IV).

Table III *Risk factors for infection caused by ampicillin resistant* Enterococcus faecium (*ARE*) *in 123 patients compared to 123 matched patient controls at Haukeland University Hospital during 1995 and 1996. Final results of backward stepwise conditional logistic regression analysis of selected variables from Table II*

Factors*	OR	Approx. 95% CI	*p*-value Likelihood ratio test
Days in hospital	1.04	1.01–1.07	0.0002
Underlying gastrointestinal disease	3.36	0.93–12.2	0.0481
Underlying neurological disease	33.5	0.74–15.30	0.0107
Days on antimicrobial treatment	8.99	1.90–42.5	0.0005
Prescription of cephalosporins	4.69	1.18–18.6	0.0147

*The following factors were also included in the starting model for the stepwise analysis: cardiovascular diseases, endocrinological diseases, haematological and solitary cancers, infections other than ARE, urogenital diseases, other diseases than the classified, number of antimicrobial agents prescribed, prescription of fluoroquinolones, metronidazole, glycopeptides, penicillins alone or combination with aminoglycosides, surgical procedures performed, urinary catheterization, central venous catheter and intrahospital death, urinary catheterization. Only factors with $P < 0.05$ were retained in the final model.

Table IV *Comparison of 123 patients infected with ampicillin resistant* Enterococcus faecium (*ARE*) *and 123 patient controls with respect to severity of underlying diseases according to McCabe and Jackson*[31]*

ARE cases	Controls			Total
	Non-fatal	Ultimately fatal	Rapidly fatal	
Non-fatal	56	15	4	75
Ultimately fatal	21	13	2	36
Rapidly fatal	10	2	0	12
Total	87	30	6	123

*The differences were not statistically significant (McNemar's test: $P = 0.3116$).

Risk factors for intrahospital death

The ARE patients dying in hospital (fatal ARE cases) were compared to those discharged alive (non-fatal ARE cases) (Table V). The different groups of underlying diseases did not represent significant risk factors for intrahospital death. However, the severity of underlying diseases was a significant risk factor in that non-fatal diseases represented a low risk for intrahospital death (OR = 0.33) whilst rapidly fatal diseases represented an increased risk (OR = 3.69) as shown in Table V. Ultimately, fatal diseases were not a significant risk factor for intrahospital death. Prescription of glycopeptides, carbapenems or a combination of penicillins with aminoglycoside prior to the diagnosis of ARE, contributed statistically significantly to death as judged by unadjusted analysis (Table V). Use of other specified antimicrobial agents or specified medical procedures did not represent any significant risk for death. In a logistic regression analysis starting with the variables shown in Table II, prescription of carbapenems was the only independent factor contributing (OR = 5.64, 95% CI = 2.03–15.67, $P = 0.0009$).

Discussion

The ARE outbreak at Haukeland University Hospital is the largest nosocomial outbreak caused by enterococci that has been described in the Nordic countries. One outbreak caused by ARE has been described from Sweden,[12] and a multiclonal outbreak caused by VRE has been reported from Finland.[38] The prevalence of ARE, unlike many other western countries, has been low in Norway until now. Therefore it is important to detect and classify the patterns of spread of these bacteria in different settings. It should be ideal to study the modes of

Table V *Unadjusted analysis of possible risk factors for intrahospital death in 23 dying patients compared to 100 surviving patients, all infected with ampicillin resistant* Enterococcus faecium (*ARE*)

Factor*	Fatal ARE cases $N=23$	Non-fatal ARE cases $N=100$	Odds ratio (OR)	95% confidence intervals	Fisher exact test *P*-value
Female gender	11	60	0.61	0.25–1.52	0.351
Days in hospital >7	22	84	4.19	0.53–33.35	0.192
Underlying diseases					
cardiovascular	3	13	1.00	0.26–3.86	1.000
gastrointestinal	6	15	2.00	0.68–5.90	0.224
endocrinological	2	9	0.96	0.19–4.79	1.000
haematological cancer	6	14	2.17	0.73–6.44	0.207
solitary cancer	5	16	1.46	0.47–4.50	0.542
infections other than ARE	9	39	1.01	0.40–2.55	1.000
urogenital	1	11	0.37	0.05–3.00	0.461
immunological	2	7	1.27	0.25–6.53	0.675
Severity of underlying diseases					
non-fatal	9	66	0.33	0.13–0.84	0.031
ultimately fatal	9	27	1.74	0.67–4.48	0.047
rapidly fatal	5	7	3.69	1.05–12.93	0.050
Prescription of antimicrobial agents					
treatment >10 days	15	68	0.88	0.34–2.30	0.809
number of agents >2	18	78	1.02	0.34–3.04	1.000
penicillins	12	48	1.18	0.48–2.98	0.818
cephalosporins	11	55	0.75	0.30–1.86	0.644
carbapenem	10	12	5.64	2.03–15.67	0.001
fluoroquinolones	5	32	0.59	0.20–1.73	0.451
aminoglycosides	10	29	1.88	0.74–4.78	0.216
metronidazole	6	18	1.61	0.56–4.65	0.389
glycopeptides	5	5	5.28	1.39–20.18	0.020
co-trimoxazole	1	13	0.30	0.03–2.50	0.465
clindamycin	2	10	0.86	0.18–4.21	1.000
penicillins and aminoglycoside	9	17	3.14	1.17–8.42	0.026
Medical procedures					
surgery	8	21	2.01	0.75–5.37	0.179
urinary catheterization	8	48	0.58	0.23–1.48	0.353
central venous line	10	26	2.19	0.86–5.60	0.127

*Factors with values not permitting calculation of odds ratio (OR) are omitted from the table.

spread and possible strategies for prevention in such a low incidence setting. As a first step in this strategy we performed a case-control study to find possible risk factors for infection.

Only few case-control studies of risk factors for ARE infection have been published. In a study from the United States[20], nine patients infected with ampicillin resistant *Enterococcus raffinosus* were compared to patients infected with ampicillin susceptible enterococci. Prior treatment with antibiotics and prolonged hospitalization were more frequent among the patients with resistant enterococci than among the controls. In another study, Venditti *et al.*[22] compared 17 ARE-infected patients (of which 16 had *E. faecium* infections) with 64 patients with ampicillin susceptible enterococcal infections. They concluded that hospitalization in a surgical service, prolonged hospital stay and prior treatment with antimicrobial agents were significant risk factors for ARE infection. None of these studies utilized multivariate analyses. Many of the possible risk factors were obviously linked to each other. For example, prescription of certain broad-spectrum antimicrobial agents may reflect severe infections not responding to other treatment, and subsequently raise the number of antimicrobial agents prescribed and the number of days on antimicrobial treatment. Therefore, univariate analyses are not reliable for complex situations like this.

In an effort to assess risk factors both for colonization and infection with ARE, Sexton *et al.*[21] performed a logistic regression analysis of 44 patients colonized or infected with ARE, compared with 100 control patients with ampicillin susceptible strains.

They adjusted their analyses for age and site of infection and found that patients with ARE were more likely to have been admitted previously to the same hospital and to have received third generation cephalosporins or clindamycin. However, only advanced age and clindamycin use were independently associated with the presence of ARE.

Risk factors for VRE infection have been studied in more detail, but most of the case-control studies are limited to rather small groups of patients. Reported risk factors for VRE infection include antimicrobial exposure, number of antimicrobial agents, days of antimicrobial use, specific agents (third generation cephalosporins, clindamycin, and imipenem), patient age, length of hospitalization, severity of underlying illness, use of electronic rectal thermometers, enteral feedings, environmental contamination and contamination of the hands of health care workers.[23] Studies on risk factors for colonization or infection with aminoglycoside resistant enterococci are also limited and the conclusions include the use of third generation cephalosporins as a significant risk factor.[39]

The choice of control group is essential for evaluation of possible risk factors. In studies where the ARE-infected have been compared to patients infected with susceptible enterococci[21,22,40] the risk factors assessed would most likely represent risk factors for selection of resistant strains. We wanted to detect risk factors for ARE infection, not only the selection of patients with resistant strains, and, if possible, plan intervention on the basis of these findings. Our choice was therefore control patients without infections. Colonization pressure is also a variable that may have an impact on the evaluation of risk factors. The colonization pressure in the study period has been assessed to be maximally 20%, measured as prevalence of faecal carriers of ARE.[19] According to the findings of Bonten *et al*. for VRE[41], the colonization pressure does not affect other risk factors for VRE significantly until it reaches 50%.

The present study, restricted to patients infected with ARE compared with patients without enterococcal infections, is large compared to the case-control studies presented previously and allows the application of multivariate analysis for risk factors. The matched design in this study ascertained that the cases and controls were comparable in respect of gender, age, hospital ward and time of entry. The most striking difference found was the mortality. The mortality in the ARE-group was more than twice as high as in the controls. Comparison of the mortality within the groups with different infection types did not reveal any significant differences (data not shown). We found a higher frequency of discharge diagnosis of haematological malignancy, infectious diseases and diseases in the central nervous system among the ARE cases and this may contribute to the higher mortality. However, comparison of the cases and controls according to the severity of underlying diseases, classified as non-fatal, ultimately fatal and rapidly fatal[31–33] did not reveal significant differences between the cases and the controls. We did also consider matching for underlying diseases, but with such an algorithm we would not be able to include a sufficient number of matched pairs to make reasonable analysis. The mortality in the ARE group in this study of 18.7% is comparable to reports from other studies.[5,21,22,40] An excess mortality of 8.9% for ARE cases demonstrated in the current study is in accordance with the 4.7% lethality for ARE infection in this outbreak which has been described elsewhere.[14] We conclude that nosocomial enterococcal infections are a significant clinical problem and also contribute to death in our hospital setting.

Both neurological and gastrointestinal diseases seemed to be significant risk factors for ARE infection, as judged by significance testing (OR = 33.5 and 3.36, respectively, Table III). However, the confidence intervals for both diagnoses included the value 1 and thus indicated that none of them were significant. Concerning neurological disease, 11 of these 17 patients had a urinary catheter, several of them were long-term patients, and all but three had been treated with antimicrobial agents (data not shown). The analysis indicates that a specific additional factor may have contributed. This factor was not the location in the hospital as they were hospitalized in eight different wards.

A possible explanation for diseases affecting the gastrointestinal tract as a risk factor for ARE infection may be that such diseases will affect colonization resistance and facilitate colonization with ARE in a way comparable to that reported for *Clostridium difficile* colonization.[23,42] Such a colonization of the gastrointestinal tract may be the first step in a clinical infection.

All types of antimicrobial agents, with the exception of narrow spectrum penicillins and ampicillins, were significantly more frequently used by the ARE patients than the controls. This is consistent with the findings of others showing that several antimicrobial agents are risk factors for ARE infection.[21,22,40] In a logistic regression analysis, we were

able to detect the use of cephalosporins as a highly significant risk factor with an odds ratio of 4.69. We were not able to demonstrate significant differences between first, second or third generation cephalosporins (data not shown).

The highly significant finding of use of cephalosporins as a risk factor is in accordance with the findings of other investigators concerning ARE, VRE and high-level aminoglycoside resistant enterococci, independent of the control groups used.[21,39,41] We suggest two possible explanations for this. First, enterococci are resistant to cephalosporins, and cephalosporins are therefore most likely to represent an effective selection pressure for enterococci. However, it is difficult to explain the difference found in studies which compare ARE patients with patients with non-ampicillin resistant enterococcal infections as a consequence of selection, unless the ampicillin-resistance also makes the enterococci even more resistant to cephalosporins. The second explanation is that severely ill patients are more prone to be treated with broad-spectrum antimicrobial agents and also seem to be more prone to be colonized with ARE, VRE or aminoglycoside resistant enterococci. It has previously been shown that the use of imipenem may be a risk factor for non-epidemic ARE infections.[40] Our finding that use of carbapenems was the only independent risk factor for death may indicate that carbapenems strongly contribute to selection of enterococci, especially ampicillin resistant enterococci. However, it is also possible that consumption of carbapenems is an indicator of severely ill and dying patients, parallel to what we suggested as an alternative explanation for cephalosporin consumption.

Our finding that prescription of antimicrobial agents for more than 10 days was a highly significant risk factor (OR = 8.99) has not been shown by others. Venditti *et al*.[22] found prior use of antimicrobial agents to be a risk factor. However, we found that use of antimicrobial agents for more than 10 days had greater impact on the risk than the effect of time as a continuous variable (data not shown). Furthermore, an independent risk factor was the exposure time in hospital, regardless of antimicrobial agents, measured as number of days in hospital. The odds ratio of 1.04 for each day (Table III) means that 10 days represents an odds ratio of 1.5 and 30 days an odds ratio of 3.2. Venditti *et al*.[22] also found that length of hospitalization was a risk factor for ARE infection.

From our model, we conclude that three factors are possible points for intervention of nosocomial ARE infection: reducing the prescription of cephalosporins, not prescribing antimicrobial agents for longer than absolutely necessary, and reducing the length of hospital stay as much as possible.

Acknowledgement

This study was supported by a grant from the Norwegian Research Council.

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