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Short communication

Antimicrobial resistance in *Escherichia coli* and *Pseudomonas aeruginosa* from Intensive Care Units in The Netherlands, 1998–2005

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Abstract

In 1998, a nationwide surveillance of antibiotic resistance among *Escherichia coli* and *Pseudomonas aeruginosa* isolates of patients from 14 Intensive Care Units in The Netherlands was initiated. Minimal inhibitory concentrations (MICs) of broad-spectrum penicillins with and without β-lactamase inhibitors, cephalosporins, aminoglycosides and fluoroquinolones were determined by a broth microdilution method. An increase in percentages of resistance of *E. coli* and *P. aeruginosa* to most antibiotics tested was observed, but rates were still lower than those described in other countries. For *E. coli*, resistance to amoxicillin was fairly stable at 44% until 2004 and increased to 56% ($P = 0.01$) in 2005. Similarly, piperacillin had a resistance rate of ca. 11% until 2004, which then increased to 38% in 2005 ($P < 0.001$). The MIC distributions of piperacillin and piperacillin/tazobactam for *P. aeruginosa* were almost identical, as were the resistance rates (4–14%). Resistance to ciprofloxacin nearly doubled in 2005 compared with previous years. Changes in resistance to the antibiotics tested were confirmed by trend analysis. Together with infection control measures, antibiotic resistance surveillance is an important tool to control the antibiotic resistance problem.

Keywords: Gram-negative rods; β-lactam antibiotics; Fluoroquinolones; Aminoglycosides; Susceptibility

1. Introduction

A worldwide increase in antibiotic resistance among Gram-negative and Gram-positive bacteria has been observed, especially in isolates derived from Intensive Care Unit (ICU) patients [1].

Appropriate therapy during the first 48–72 h after admittance to the ICU is crucial for a positive outcome [2]. Empirical therapy in The Netherlands comprises mostly a combination of a β-lactam antibiotic (broad-spectrum penicillin or second- and third-generation cephalosporin) with an aminoglycoside, whereas carbapenems, fourth-generation cephalosporins and ciprofloxacin may be used as alternatives in some situations. Knowledge of the resistance level of these antibiotics is crucial for adequate (empirical) antibiotic therapy. Therefore, in 1998 an antibiotic resistance surveillance programme on specific wards in Dutch hospitals was initiated. This study reports the results for β-lactam antibiotics, aminoglycosides and ciprofloxacin in two indicator organisms, *Escherichia coli* and *Pseudomonas aeruginosa*, from ICU patients admitted to 14 large hospitals in The Netherlands between 1998 and 2005.

2. Materials and methods

2.1. Setting

The surveillance programme started in 1998 and involved patients from adult ICUs of two university hospitals and 12 large referral hospitals from across The Netherlands. More than 25% of the Dutch population was covered by these hospitals.

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2.2. Isolates

Yearly, from January to July, 100 unique, unrelated, consecutive clinical isolates, including *E. coli* and *P. aeruginosa*, of ICU patients were collected in each of the 14 hospitals. Only one isolate per patient and species was included. The strains were identified at the local laboratory, kept at −20 °C and sent to the central laboratory for batch-wise antimicrobial susceptibility testing.

2.3. Susceptibility testing

Minimal inhibitory concentrations (MICs) were determined by the broth microdilution method with Mueller–Hinton II cation-adjusted broth (Becton, Dickinson and Company, Sparks, MD) according to the Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Laboratory Standards) guidelines, as previously described [3,4]. Microtitre plates containing freeze-dried antibiotics were obtained from MCS Diagnostics BV (Swalmen, The Netherlands) after quality testing by the manufacturer and guarantee of a maximum shelf-life of ca. 1 year.

The antibiotics tested were (concentration range in mg/L): amoxicillin (0.06–128); amoxicillin/clavulanic acid (co-amoxiclav) (ratio 4:1) (0.06–128); piperacillin (0.25–512); piperacillin/tazobactam (0.25/4–512/4); cefuroxime (0.06–128); ceftazidime (0.06–128); cefotaxime (0.06–128); cefixime (0.06–128); ceftibuten (0.06–128); cefepime (0.12–128); imipenem (0.06–64); meropenem (0.03–64); gentamicin (0.03–64); tobramycin (0.06–128); amikacin (0.12–256); and ciprofloxacin (0.008–16). The MIC was defined as the lowest concentration showing no growth in the microtitre plates after 18 h of incubation at 37 °C.

Breakpoints for resistance were as defined by the CLSI [3]. *Escherichia coli* ATCC 35218 and ATCC 25922 were used as control strains.

The central laboratory used the same method throughout the whole study period.

2.4. Statistical analysis

A Mann–Whitney *U*-test was used to compare susceptibility percentages for individual antibiotics between different years of surveillance. A logistic regression analysis was performed to determine statistically significant trends over time in changes of resistance of the isolates to the antibiotics tested during the study period, adjusted for participating centres. A *P*-value of <0.05 was considered statistically significant.

3. Results

In total, 1267 *E. coli* and 741 *P. aeruginosa* isolates were included.

3.1. Escherichia coli

Amoxicillin resistance fluctuated at around 44% from 1998 to 2004 (Fig. 1a), but increased to 56% ($P = 0.01$) in 2005. Trend analysis using a logistic regression model confirmed the increase in resistance (from 42% to 51%; $P < 0.001$). The distribution of MICs in 1998 and 2005 (Fig. 2a) showed two subpopulations: a susceptible one with a broad MIC range from 1–8 mg/L and a highly resistant one with MICs >32 mg/L. A shift was observed in the susceptible subpopulation, with more strains inhibited by 8 mg/L in 2005 compared with 1998. Similarly, in the resistant subpopulation more strains with MICs >32 mg/L (mostly >256 mg/L) were observed in 2005 ($P < 0.05$) compared with 1998.

Co-amoxiclav resistance also fluctuated. It showed an increase to 22% in 2000 with a decrease to 8% in 2001 and thereafter an increase to 22% in 2005 (Fig. 1a). Trend analysis confirmed the overall increase (from 13% to 18%; $P = 0.001$). The MIC distribution showed a highly resistant subpopulation (MICs > 32 mg/L, 9% of the total population) and a growing percentage of strains (up to 40%) with MICs ≥ 16 mg/L in 2005 (Fig. 2b).

The overall resistance level of piperacillin was ca. 11% until 2004, which increased to 38% in 2005 ($P < 0.001$) (Fig. 1a). Trend analysis confirmed the increase in resistance (from 4% to 32%; $P < 0.001$). The MIC distributions of piperacillin (Fig. 2c) showed three subpopulations: one susceptible (MICs = 0.5–4 mg/L), one moderately susceptible (MICs = 8–64 mg/L) and one resistant subpopulation (MICs > 64 mg/L). Piperacillin showed higher activity than amoxicillin towards the same population: the peak of piperacillin MICs in the susceptible range was at 1–2 mg/L compared with 4 mg/L for amoxicillin (Fig. 2a and c).

Cefuroxime resistance fluctuated during the study period from 1% to 12%, with a mean of 6%; no increase was observed (Fig. 1b). Trend analysis even showed a slight decrease in resistance (from 7% to 5.5%; $P = 0.029$).

Cefotaxime and ceftazidime showed stable resistance levels of ca. 2% and 1%, respectively. The rates for ceftibuten and cefepime resembled those of cefotaxime and ceftazidime, respectively, and that of cefixime was comparable with that of cefuroxime (5.5% in 2005; Fig. 1b). Trend analysis showed no significant changes in resistance, except for cefixime with an increase to 5% ($P = 0.024$), i.e. similar to the level of cefuroxime resistance. The MIC distribution of cefuroxime (Fig. 2e) showed a marked difference between 1998 and 2005, with a shift towards lower MIC values in the latter year. Cefotaxime, ceftazidime and cefepime showed a unimodal distribution in 2005 over a very small range, with 90% of strains having MICs ≤ 0.25 mg/L. Occasionally, resistant strains were recorded. The MIC distributions in 2005 of ceftibuten and cefixime ranged over a broader area, from 0.06–4 mg/L, with a MIC for 90% of the organisms of 1–2 mg/L (Fig. 1c).

Gentamicin resistance fluctuated between 1% and 4% (mean 3%) until 2004 and showed an increase to 7% in 2005