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MICROBIOLOGY

Active surveillance for multidrug-resistant Gram-negative bacteria in the intensive care unit

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Summary

A short-term program of performing serial active screening cultures (ASC) in the intensive care unit was instituted to establish a method for the detection of antibiotic-resistant Gram-negative bacteria (GNB) and the local rates of colonisation. Of all submitted ASC, 25.9% (30/116 collected swabs) isolated an antibiotic-resistant GNB. ChromID ESBL agar (bioMérieux, France) identified the majority of these organisms, with the additional antibiotic-impregnated media [MacConkey agar (MCA) with ciprofloxacin, MCA with gentamicin and MCA with ceftazidime] adding limited benefit. Compared to swabs performed on admission, 37.8% (14/37) of patients cultured a new antibiotic-resistant isolate on discharge. Serial screening in intensive care has the ability to identify patients with unrecognised colonisation with antibiotic-resistant GNB; however, the increase in the laboratory workload and logistical challenges in the collection of the surveillance swabs may limit this program's expansion.

Key words: Active screening culture, active surveillance, antibiotic resistance, intensive care unit, rectal swab, multidrug-resistant Gram-negative bacteria.

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INTRODUCTION

Patients colonised with antibiotic-resistant Gram-negative bacteria (GNB) represent a reservoir of organisms that can precede invasive disease and spread to other patients.[1,2] Colonisation may be only detected by performing surveillance cultures,[3] and has been reported to precede bacteraemia with the same species in the week prior.[4–6] Colonisation is prolonged and microbiologically diverse,[7] and detection is more complex than for other resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* species (VRE).[8] The European Society of Clinical Microbiology and Infectious Diseases recommends implementing a program of active screening cultures (ASC) at hospital admission followed by contact precautions to reduce the spread of antibiotic-resistant GNB [extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae*, multidrug-resistant (MDR) *Klebsiella pneumoniae*, MDR *Acinetobacter baumannii* and MDR *Pseudomonas aeruginosa*] in the epidemic setting.[9] In the endemic setting, they recommend implementation of ASC only as an additional measure if basic measures fail to control the spread of MDR GNB. The Australian Commission on Safety and Quality in Health Care Multi Resistant Gram-negative Taskforce recommend actively screening for carbapenem-resistant *Enterobacteriaceae* (CRE) in patients with overseas hospital contact in the past 12 months, people who are identified as a CRE contact during their hospitalisation, and patients with past demonstrated CRE colonisation or infection.[10] This study aims to pilot an ASC program to detect multidrug-resistant bacteria and establish the current rates of colonisation on admission to and discharge from the intensive care unit (ICU).

MATERIALS AND METHODS

The study was approved by the Ethics Committee (Alfred Health Research and Ethics Unit, Melbourne, Australia). Patients were enrolled in a prospective observational cohort study over a 3-month period, October to December 2011, in a 45-bed ICU serving as a quaternary referral centre, caring for a complex case load of adult patients, including heart and lung transplantation, bone marrow transplantation, extra-corporeal membrane oxygenation, burns and adult trauma. Local empiric sepsis guidelines recommended piperacillin-tazobactam and gentamicin, or ciprofloxacin (depending on patient co-morbidities), and vancomycin (if prior hospitalisation >7 days). In order to assess the impact of ICU stay on colonisation with antibiotic-resistant GNB, only patients who stayed in ICU ≥5 days were planned to be screened. All new admissions to ICU in the previous 48 h were assessed on a daily basis (Monday to Friday) for enrolment. Patients were excluded if they were <18 years old, or were admitted to ICU following uncomplicated surgery (e.g., coronary artery bypass, other vascular or intra-abdominal surgery, etc.) or minor trauma, or if the ICU admission diagnosis was any other condition that would mean they would be unlikely to spend ≥5 days in the ICU. Limited demographic data were collected, including age, gender, ICU length of stay, hospital length of stay, location prior to ICU admission (community or hospital ward) and location within ICU that the patient was admitted to (cardiac, trauma or general ICU sections).

Rectal swabs (with Amies agar gel transport medium; Copan Italia, Italy) were collected on admission to ICU (collected within the first 48 h) and discharge from ICU (collected either on the day of discharge from ICU or in the following 24 h after discharge from ICU). Written information and instructions on how to correctly perform the screening swab was provided to the nursing staff and the patient, where appropriate. Verbal consent was obtained prior to swab collection by the nursing staff concordant with existing standard practice for collecting VRE screening swabs. Where the patient was intubated and unable to give consent, a waiver for the requirement of obtaining individual consent was granted by the Ethics Committee. A moistened rectal swab was inserted past the anal sphincter, rotated at least one full turn, withdrawn and placed into the transport tube. Swabs were first inoculated in enriched heart infusion broth overnight. The broth was assessed the following day for turbidity, streaked onto selective media and then re-incubated for a further 24 h. Five selective agar plates were used: MacConkey agar (MCA), MCA with gentamicin 8 mg/L, MCA with ciprofloxacin 2 mg/L, MCA with ceftazidime 2 mg/L and ChromID ESBL (bioMérieux, France). If there was no growth identified on the MCA after 24 h, then the broth was streaked onto non-selective horse blood agar (HBA) such that any organism growth would indicate that adequate sampling

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had occurred. Any growth on selective media underwent identification by Vitek2 (bioMérieux). Antibiotic resistance was determined by disc diffusion for the following antibiotics: ciprofloxacin (5 μg), gentamicin (10 μg), piperacillin-tazobactam (30/6 μg), ceftriaxone (30 μg), ceftazidime (10 μg) and meropenem (10 μg) (interpretation by European Committee on Antimicrobial Susceptibility Testing, version 1.3, 2011). Phenotypic beta-lactamase production was performed to detect extended spectrum beta-lactamase (ESBL) production, by double disc synergy testing,[11] and AmpC production by boronic acid disk test when there was a reduced zone of inhibition to cefoxitin 30 μg disc.[12,13] Culture results were made available to the treating ICU clinicians on request. If a carbapenem-resistant isolate was identified, however, the hospital Infection Prevention Unit would be automatically notified such that contact precautions could be implemented. Other beta-lactamase producing isolates or MDR GNB were not isolated. Resistance to any of the tested antibiotics defined that the isolate was an 'antibiotic-resistant GNB'. MDR was defined as non-susceptible to greater than or equal to three of the following antimicrobials: (1) piperacillin-tazobactam; (2) meropenem; (3) ceftriaxone or ceftazidime; (4) ciprofloxacin; (5) gentamicin.[7,14] Beta-lactamase encoding genes were assessed by polymerase chain reaction (PCR) from purity cultures, including $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{CMY}, bla_{TEM} as previously described.[15,16] Clonal relatedness was investigated by repetitive sequence-based PCR (Rep-PCR) and analysed using DiversiLab (bioMérieux).[17] Statistical analysis of the paired data from patients swabbed both on admission and discharge was assessed with McNemar's test.

RESULTS

A total of 110 patients were identified as eligible for enrolment by the treating ICU doctors. Thirty-one patients did not have active screening cultures collected and were not enrolled. Of the remaining 79 patients who were screened on admission, 37 patients were swabbed also on discharge from ICU (Fig. 1). Of the 42 patients that were screened only on admission, 24 left ICU or died prior to staying 5 days. Of the 79 enrolled patients, the mean age was 55 years (standard deviation 19 years) with a male predominance (70.9%). Three-quarters of the patients (59, 74.7%) were admitted directly to ICU from the community and the remainder were admitted from a hospital ward. Half of the patients were admitted to the trauma section of the ICU (40, 50.6%). The others were split between the general (24, 30.4%) and cardiac (15, 19.0%) sections. For the 37 patients who were swabbed twice, the median number of days between swabs was 10 days (interquartile range 8–17 days).

Of the 116 screening swabs collected, antibiotic-resistant GNB were identified in just over a quarter (30 swabs, 25.9%). Antibiotic susceptible GNB, detected by growth on the plain MCA only, occurred in over half of the swabs (66, 57.0%). No growth of any GNB on the screening media occurred in 20 swabs (17.2%), however all incubated broths subsequently demonstrated growth when streaked on non-selective HBA. ChromID ESBL agar supported the growth of the majority of antibiotic-resistant GNB, with exception of three *Escherichia coli* isolates that were only ciprofloxacin and/or gentamicin-resistant, and an *Enterobacter cloacae* isolate that grew only on MCA with ceftazidime. Of the 26 swabs that grew on ChromID ESBL agar, the MCA with ceftazidime supported the same growth except for three swabs that isolated ESBL isolates (2 *E. coli* and 1 *Klebsiella oxytoca*) and one swab that isolated *Citrobacter freundii*. There was minimal breakthrough growth of susceptible GNB isolates, which when present demonstrated antibiotic-affected colony morphology on the MCA with impregnated antibiotics. Susceptible *Pseudomonas* isolates were also noted to occasionally breakthrough on the ESBL ChromID agar.

The proportion of patients who isolated any type of antibiotic-resistant GNB on the ICU admission ASC was 16.5% (13/79 patients), of which five were screened again on discharge, and four re-isolated the same organism. Colonisation with antibiotic-resistant GNB increased following ICU admission, especially beta-lactamase producing isolates that increased five-fold (Table 1). Of the 37 patients who were screened twice, 14 (37.8%) isolated a new antibiotic-resistant GNB compared to their admission swab, of which three patients cultured more than one new antibiotic-resistant GNB (ESBL *K. pneumoniae* and *E. cloacae*; ESBL *K. pneumoniae* and ciprofloxacin-gentamicin-resistant *E. coli*; ESBL *K. pneumoniae*, *E. cloacae* and *Acinetobacter baumannii*). No isolate was found to be carbapenem resistant, or demonstrated a reduced zone of inhibition to warrant carbapenamase phenotypic or genotypic testing (<25 mm diameter with a meropenem 10 μg disk). There was no correlation between colonisation status and ICU length of stay, age, sex or location prior to ICU admission, or location within ICU.

A *Klebsiella pneumoniae* isolate (ESBL-producing and ciprofloxacin-resistant) was identified in one of 37 patients on admission (who was admitted to ICU from a hospital ward) compared with six of 37 patients on discharge. All isolates shared the same antibiogram and molecular mechanisms of resistance (bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M}}$). Rep-PCR analysis

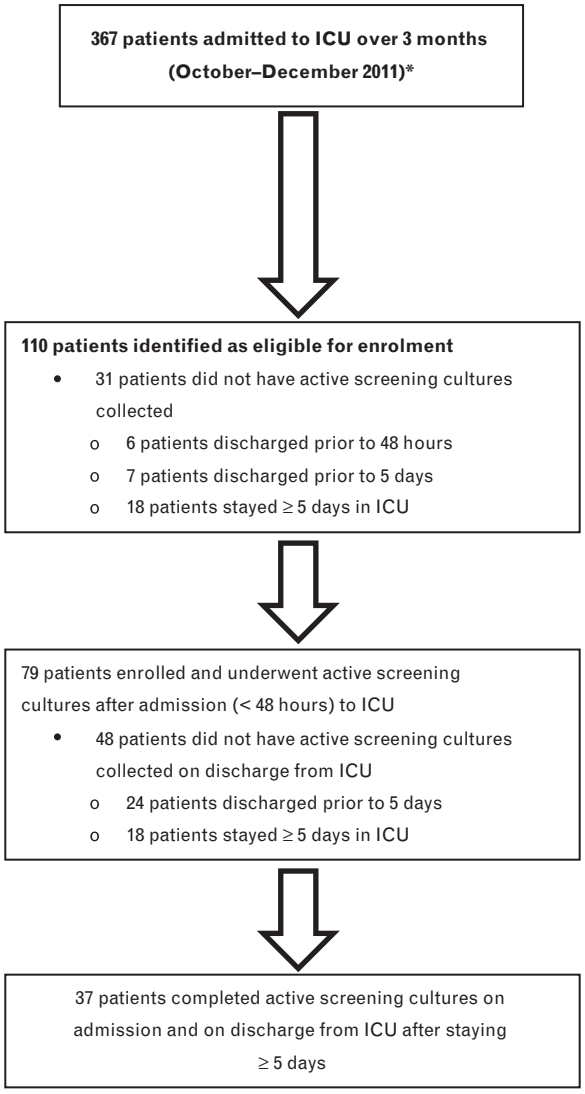

Fig. 1 Flow chart for patient enrolment and active screening culture collection. *A total of 113 patients of all the ICU admissions in the 3 month period went on to have an ICU length of stay ≥ 5 days.