J Infect Chemother 22 (2016) 450–455

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original article

Sources of sporadic *Pseudomonas aeruginosa* colonizations/infections in surgical ICUs: Association with contaminated sink trap

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ARTICLE INFO

Article history:
Received 24 November 2015
Received in revised form
1 March 2016
Accepted 31 March 2016
Available online 14 May 2016

Keywords:
Intensive care unit
Pseudomonas aeruginosa
Sink
PFGE
Waterborne infections

ABSTRACT

Background: Many studies have reported the hospital outbreaks of *Pseudomonas aeruginosa* due to cross-contamination between patients and water fittings, but the importance of water fittings as sources of sporadic *P. aeruginosa* colonizations/infections remains ambiguous.
Aim: To investigate the sources of sporadic *P. aeruginosa* colonizations/infections in a clinical intensive surveillance, and further analysis the potential of sink trap for *P. aeruginosa* transmission in intensive care units (ICUs).
Methods: Patients monitoring and targeted environmental screening for *P. aeruginosa* was performed prospectively over a 27-week period, in absence of recognized outbreak, in two surgical intensive care units (SICUs). All isolates were genotyped by Pulsed field gel electrophoresis analysis.
Findings: 18.9% (46/244) of water fitting samples harbored *P. aeruginosa*, and active screening samples from 9.2% (55/595) of hospitalized patients carried with *P. aeruginosa*. According to genotype results, approximately 50% of *P. areuginosa* colonizations/infections of patients were of exogenous origin. 64.7% (11/17) of exogenous sourced cases were associated with contaminated sink traps. There was a significant correlation between the incidence of exogenous colonization/infection and the prevalence of *P. areuginosa* in water fitting in SICU-2 ($r_s = 0.972$; $p = 0.014$). Furthermore, *P. areuginosa* from sink trap possessed a higher level of resistance to multi-antibiotics as opposed to cross-transmission from other patients.
Conclusion: Water fitting especially sink trap act as an important role in sporadic *P. aeruginosa* transmission in SICU patients. This report highlights the necessity of identification of potential environmental reservoirs, such as sinks, for control of infections of environmentally hardy multi-resistant *P. areuginosa*.

1. Introduction

Pseudomonas aeruginosa is a ubiquitous pathogen prevalent in humid environments that is responsible for severe hospital infections. It is associated with high morbidity and mortality in immunocompromised hosts and in intensive care units (ICUs) patients [1]. Its remarkable ability is to adapt to adverse condition with the presence of antiseptic substances and limited nutrition (e.g. water) [2,3].

Pathways of *P. aeruginosa* infections in ICUs include endogenous infection from the patient's own oropharyngeal and intestinal flora, and exogenous infection from the hands of the personnel, or from neighboring patients and environment. Contaminated water fittings, including drinking water, mineral bottled water, electronic faucet, water outlet, sink and waste-water system, have been reported as the sources of many outbreaks of *P. aeruginosa* infection in ICUs [4–9]. It is found that only seven publications provided plausible evidence of a link between a water fittings acting as a reservoir for *P. aeruginosa* and infection/colonization in patient [10]. Most of the literature is descriptive and based on outbreak reports. Furthermore, several studies suggest that exogenous transmission is uncommon during non-outbreak period [11–13]. Water fitting seems to play a smaller role in non-epidemic situations than

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http://dx.doi.org/10.1016/j.jiac.2016.03.016
1341-321X/

expected by many operational hospital hygiene teams [14]. Whereas, other studies have shown it play a key role as source of colonizations/infections using molecular typing [15,16]. Knowledge of the accurately sources and patterns of *P. aeruginosa* colonizations/infections are crucial for designing optimal infection control and prevention strategies. Thus, we prospectively monitor patients (clinical samples and active screening samples), water fittings (tap water, tap outlet, and sink trap), hands of healthcare workers (HCWs), and environmental surfaces for contamination with *P. aeruginosa* in two adult surgical intensive care units (SICUs) to elucidate the sources of sporadic *P. aeruginosa* colonizations/infections.

The importance of tap water as the source of spread of *P. aeruginosa* has been accepted by many specialists [17,18]. The clinical impact of sink traps as reservoirs of *P. aeruginosa* has not yet been fully explored. Some studies have reported outbreaks of multidrug-resistant bacteria such as extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* linked contaminated sink traps [19,20]. Furthermore, sink traps can act as cradles to the emergence of bacteria armed with abilities to resist multiple antibiotics [21]. Therefore, an in-depth epidemiological study is also necessary to investigate whether sink traps play an important role in *P. aeruginosa* transmission in ICU.

2. Materials and methods

2.1. Setting

This study was performed in two adult surgical ICUs of a 1600-bed university-affiliated tertiary care hospital during a 27-week period, from March 2011 to October 2011. No recognized outbreaks were observed during the study. The SICUs had two distinct water distribution networks and waste water systems. Shown in Fig. 1, there were 29 beds (SICU-1) and 10 beds (SICU-2) in an open room harboring 4 taps and 4 sinks. The other water sites were located in the treatment room and waste room.

2.2. Study design

This study has been approved by the ethics committee. The approval number is 2009-64. During the study, patients hospitalized in SICUs were screened for the presence of *P. aeruginosa* on admission and weekly thereafter by rectal or respiratory swab. Patients stayed in SICUs less than 72 h were excluded. In case of any suspicious infections, diagnostic specimens were collected from all possible infected sites. We monitored the environmental surfaces and hands of HCWs only if *P. aeruginosa* were isolated from diagnostic samples. Hands of HCWs were sampled only from nursing staff working directly with patients. Seven locations were selected as environmental surface sampling sites, including the surface of counter, bed rail, bed control, instrumental panel, infusion pump, medical chart, and stethoscope. At 4-weekly interval, samples from all cold tap water, tap inner surface, and sink trap were obtained.

2.3. Microbiological analysis

One hundred milliliters of cold tap water was collected into sterile bottle immediately after opening the tap. Samples were subsequently passed through sterile filter membranes (pore size: 0.45 μm; Millipore, USA). The membranes were placed on cetrimide agar plates (Oxiod, UK) at 37 °C and examined for growth of colonies after 24 h and 48 h. The swab samples, isolated from tap inner surface, sink trap, active screening samples, HCWs' hand samples and environmental surface, were directly inoculated on cetrimide agar plates. *P. aeruginosa* was identified by routine methods such as growth characteristics and biochemical fermentation. Sensitivity testing was performed using disk diffusion methodology. Antibiotics tested included carbapenems (imipenem and/or meropenem), cefazidime, cefepime, amikacin, piperacillin-tazobactam, piperacillin, cefoperazone-sulbactan, levofloxacin and aztreonam.

2.4. Molecular typing

All environmental and clinical isolates were genotyped by Pulsed field gel electrophoresis (PFGE) analysis of SpeI restricted

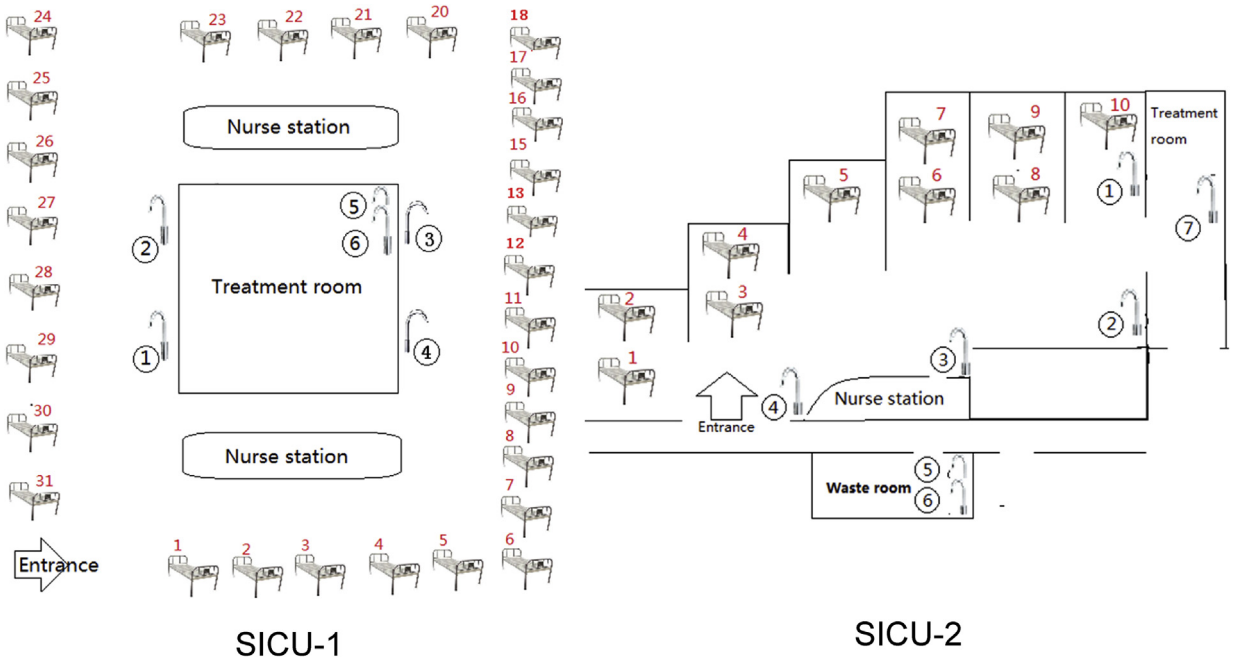

Fig. 1. The layout of two adult surgical ICUs. The number in circles was the serial number of water fittings (tap and sink).

genomic DNA [22]. PFGE was performed using a CHEF-DR III apparatus (Bio-Rad, USA) setting at 6.0 v/cm, with a linear ramping from 2.19 s to 30.82 s for 18 h. The PFGE-SpeI profiles were converted into a matrix of discrete characters and analyzed by a parsimony method. Strains showing identical SpeI PFGE profiles were defined as being individuals of a same clone.

2.5. Epidemiological definitions and classification of cases

A case was defined as the isolation of a given genotype of *P. aeruginosa* from a diagnostic specimen obtained during a patient's stay in a SICU [23]. Thus the isolates from an individual patient having two different PFGE patterns (genotypes) yielded two distinct cases. Cases were classified into four categories according to molecular typing data: (A) identical to isolates from water fittings; (B) identical to at least one other patient sample and/or environmental surface sample, but not found in water fitting; (C) identical to rectum and/or throat sample of the same patient; and (D) unique genotype. Cases in categories A and B were considered as possibly exogenous transmission, and cases in category C and D as possibly endogenous transmission.

2.6. Statistical analysis

The data were analyzed with SPSS version 16.0. Percentage of *P. aeruginosa* carriage and water fitting contamination of two SICUs were compared using the χ^2 test, a p value of less than 0.05 was considered statistically significant. The correlation coefficient of incidence of exogenous colonization/infection and the percentage of water fitting contamination with *P. aeruginosa* was calculated by using two-tailed spearman's analysis.

3. Results

3.1. Study participants

During the 27-week period, from 595 patients stayed in SICUs more than 72 h (467 to SICU-1 and 128 to SICU-2) with a total of 5674 days of hospitalization (4401 to SICU-1 and 1273 to SICU-2), thirty-two patients (21 to SICU-1 and 11 to SICU-2) presented at least one clinical diagnosis sample positive for *P. aeruginosa*. The patients with *P. aeruginosa* at admission were not counted in. The overall incidence of colonization/infection was 5.6 per 1000 patient-days of hospitalization (4.8 to SICU-1 and 8.6 to SICU-2). Most of patients were suffered from pneumonia (n = 8) or surgical wound infection (n = 9). Thirty strains were isolated from sputum samples. The others were isolated from secretion, pleural fluid, drainage fluid and blood. Moreover, active screening samples from 9.2% (55/595) of hospitalized patients harbored *P. aeruginosa* (Table 1). In total, 93 strains collected from patients were analyzed: 58 screening samples from 55 patients and 35 diagnostic samples from 32 patients.

3.2. Environmental investigation

Totally, 456 environmental samples were collected from both SICUs (244 to water fittings, 189 to environmental surfaces and 23 to hands of HCWs) (Table 2). Samples positive for *P. aeruginosa* were 18.6% (18/97) of the tap water, 22.1% (21/95) of the sink traps and 13.5% (7/52) of the tap outlets, respectively. The positive site rate of sink traps (92.3%, 12/13) was higher than that of tap water (53.8%, 7/13). Almost half of taps sites (tap water and tap outlets) were negative to *P. aeruginosa* (tap1, tap2 and tap3 in SICU-1; tap1, tap2 and tap7 in SICU-2), while all sink traps were detected with *P. aeruginosa* except sink 1 in SICU-2. No strains were isolated from the hands of HCWs. Only 5 strains were detected from two bed rails, one counter, one instrument panel, and one medical chart. In total, 53 strains from environment were collected and analyzed. Two samples taken from sink traps contained two strains.

3.3. Sink trap as a continuous exogenous source of P. aeruginosa transmission

PFGE typing distributed the 146 isolates into 89 different genotypes: 23 genotypes recovered from 35 patient strains (14 to SICU-1 and 9 to SICU-2), 53 genotypes from 58 active screening isolates, and 24 from 53 hospital environmental isolates. The strains from active screening samples of patients showed a relatively greater genetic diversity than those from environment and clinical patients.

According to epidemiological and molecular data, thirty-four cases were divided into four categories (Table 1). One patient was found to harbor three different strains (genotypes). Our data showed that 17.6% (6/34), 32.4% (11/34), and 50.0% (17/34) of colonization/infection with *P. aeruginosa* were original from other patients, water fitting, and endogenous flora, respectively. In SICU-2, a significant correlation between numbers of exogenous cases per 1000 admissions and the percentage of water fitting contamination at two-monthly intervals was observed ($r_s = 0.972$; $p = 0.014$). It is apparent that water fitting was an important part of exogenous transmission. Furthermore, 64.7% (11/17) of exogenous sourced cases were associated with contaminated sink traps. Whereas, no strains (genotypes) recovered from tap water were identical to that from patients. Fig. 2 showed the spatio-temporal

Table 1
Epidemiological data of patients with *Pseudomonas aeruginosa* stayed in surgical intensive care units (SICUs) more than 72 h (2011.3–2011.10).

Epidemiological data	SICU-1	SICU-2	Total
Number of admissions stayed in SICUs more than 72 h	467	128	595
Patient-days of hospitalization	4401	1273	5674
Number of patients colonization/infection with *P. aeruginosa*	21	11	32
Number of patients colonization/infection with *P. aeruginosa* per 1000 admissions	45.0	85.9	53.8
Number of patients colonization/infection with *P. aeruginosa* per 1000 patient-days of hospitalization	4.8	8.6	5.6
Percentage of hospitalized patients carried with *P. aeruginosa* (%)	7.7	14.8	9.2
Percentage of water fitting contamination with *P. aeruginosa* (%)	19.6	17.0	18.4
Categories of cases (number of cases per 1000 admissions)[a]			
Category A	23.6	0	18.5
Category B	4.3	31.3	11.8
Category C	4.3	15.6	6.7
Category D	17.1	39.1	21.8

[a] Category A, identical to water fitting; Category B, identical to at least one other patient and/or environmental surface sample, not found in water fitting; Category C, identical to active screening sample of the same patient; Category D, unique genotypes.

Table 2
Positive rates of *Pseudomonas aeruginosa* isolated from environment and patients (2011.3–2011.10).

Source of samples	Number of samples	Number of positive samples	Positive rates (%)
Environmental samples	456	51	11.2
Tap water	97	18	18.6
Tap outlets	52	7	13.5
Sink traps	95	21	22.1
Hands of staff	23	0	0
Environmental surfaces	189	5	2.6
Screening samples	595	55	9.2
Diagnostic samples	N/A	32	N/A

N/A: Not Applicable.

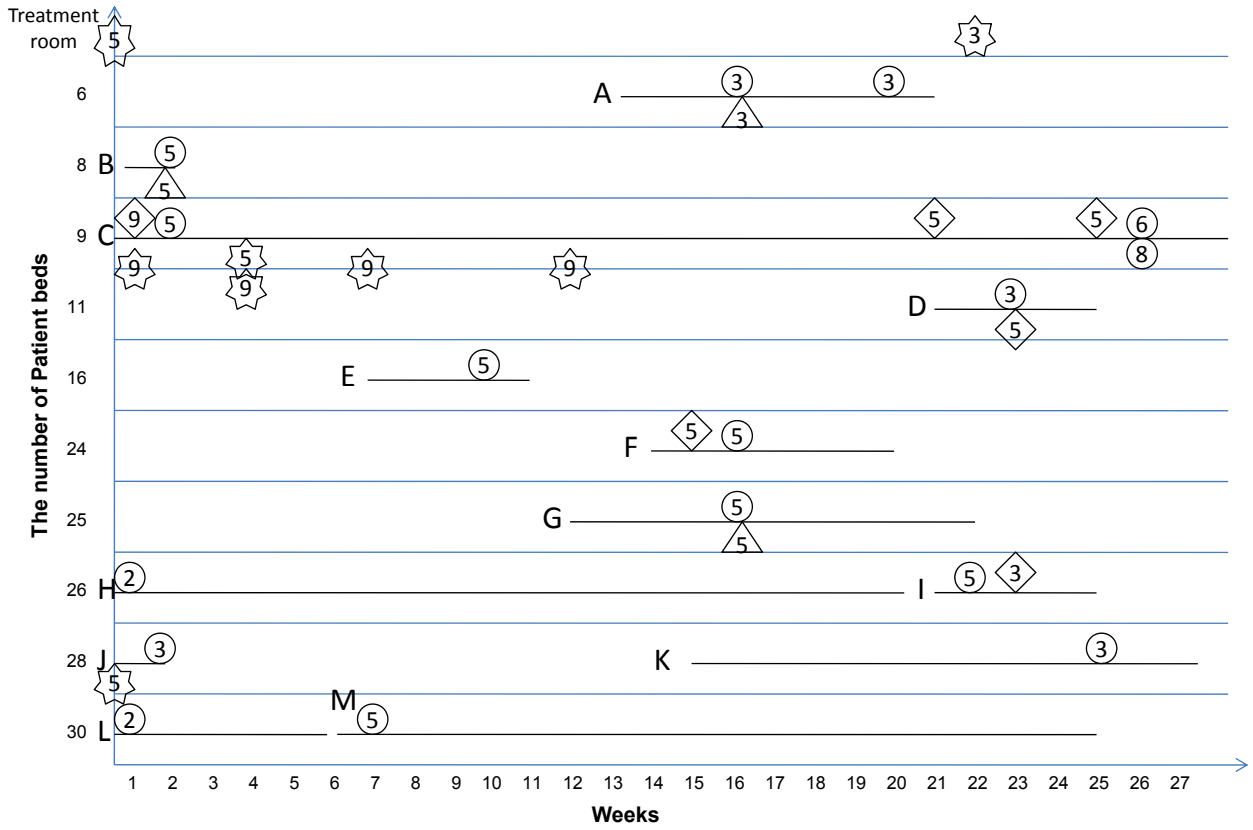

Fig. 2. Illustration of the spatio-temporal dynamics among patients with exogenous sourced *P. aeruginosa* in SICU-1. Number in circles: genotype from patients; Number in rhombuses: genotype from active screening samples (rectum); Number in starts: genotype from sink traps; Number in triangles: genotype from environmental surfaces; Horizontal line: patient's individual stay.

dynamics among patients with exogenous sourced *P. aeruginosa* in SICU-1. Thirteen patients with 6 different genotypes were included. Three genotypes recovered from patients were identical to that from sink trap samples (genotype 3, genotype 5 and genotype 9). Among these cases, cross contaminations between the patients J and A, K, D can be excluded since no overlapping hospitalization period occurred. It seemed that some vehicles such as sink trap can be contaminated and consecutively contribute to spread of *P. aeruginosa.* Four strains found on the environmental surface had the same genotype with strain from patients.

Differences in antibiotic resistance levels were observed within the isolates from diagnostic, screening, and environmental samples. The percentage of carbapenem-resistant *P. aeruginosa* of diagnostic samples (45.7%, 16/35) was higher than that of screening samples (3.4%, 2/58) and environmental samples (15.1%, 8/53). All strains from water-related samples were sensitive to all antibiotics. Some sink-related strains showed the multi-resistance profiles. Especially, patient isolates associated with sink trap showed more resistant to antibiotics than patient-to-patient transmission strains (the percentage of carbapenem-resistant *P. aeruginosa*: 81.8% vs. 16.7%).

4. Discussion

P. aeruginosa is one of the most important aetiological agents of nosocomial infections. Several studies have investigated the sources of colonizations/infections of ICU patients by *P. aeruginosa*, and showed that cross-contamination between patients and water fitting could explain several nosocomial outbreaks [4–9]. Strains involved in these outbreaks are considered as transient colonizers of the hospital units. Rarely, a *P. aeruginosa* population of a unit is analyzed outside the scope of an epidemic outbreak. Here, we initiated a long-term clinical intensive study regarding sources and

patterns of *P. aeruginosa* colonizations/infections in two surgical ICUs patients.

P. aeruginosa outbreaks sourced from sinks were observed as long ago as the early 1980s [24]. Early reports implicated the sinks may be reservoirs for large number of highly resistant *P. aeruginosa* but are rarely the source of organisms colonizing patients in ICU [25]. Since the 2000s, tap water contaminated with *P. aeruginosa* had been recognized as a major and continuous source for transmission. Recently, several studies reported the outbreaks of MDRP in hospitals, which highlight the potential of hospital waste system to act as reservoir of MDRP [9,19,26]. To our knowledge, the present study is the first to investigate the sink trap as an important source of colonization/infection of *P. aeruginosa* over a long period of time.

According our data, water fittings were found to contain a long-standing population of *P. aeruginosa*, with strains were detected in 18.6% of tap water samples and 22.1% of sink trap samples. The positive site rate of sink traps (92.3%, 12/13) was higher than that of tap water (53.8%, 7/13). It suggested that the extent of *P. aeruginosa* colonization was more seriously for sink trap than for tap water. Moreover, approximately 50% of *P. areuginosa* colonizations/infections of ICU patients were of exogenous origin, the data in line with the results of Agodi et al. [27]. 64.7% (11/17) of exogenous sourced cases were associated with contaminated sink traps. Whereas, no strains (genotypes) recovered from tap water were identical to that from patients. It also showed the positive correlation between the incidence of exogenous colonization/infection and the prevalence of *P. areuginosa* in water fitting in SICU-2 ($r_s = 0.972$; $p = 0.014$). Shown as the spatio-temporal dynamics among patients with exogenous sourced *P. aeruginosa* in SICU-1, the pattern of infection was small clusters with long infection-free intervals, suggesting that person-to-person spread did not play a major role in the infection. Together these findings implicated that water fitting especially sink trap can be an important and continuous source of exogenous colonizations/infections. *P. aeruginosa* transmission from sink trap to patients may be initiated by splashing back the organisms residing down the trap onto staff hands or patient areas when staffs wash hands or something is poured into the basin.

Interestingly, clinical isolates associated with the sink trap (as opposed to cross transmissions from other patients) possessed a higher level of resistance to multi-antibiotics. Several factors probably enhanced the development of resistance. More bacteria and more antibiotics are flushed down the sink due to the nature of hospitals caring for patients that are ill and treated with antibiotics. Thus, biofilm in building drains provided the cradle to the transfer of antibiotic resistance between different bacteria [28].

Several limitations of our study must be mentioned. Firstly, Only 34 cases were included in this observational study. A case–control study or cohort study with abundance of cases should be performed in the future. Secondly, the limitation of sampling technique and microbiological analysis of water fitting samples may underestimate the role of water fitting. Some studies typed up to 4–10 different colonies from each culture [29]. Just one to two colonies from a culture were picked and genotyped in our study. Therefore, more correlations between sink trap and patient isolates might have been detected. At last, we did not analyze various sources from equipment and might have underestimated the number of exogenous source.

In conclusion, sink trap can serve as a reservoir for waterborne pathogens and act as the source of *P. aeruginosa* transmission in ICUs patients under non-outbreak situations. If we find *P. aeruginosa* in the sink, we may find colonization/infection in patients. Therefore, appropriate sinks disinfection or maintenance should be adapted actively for preventing the *P. aeruginosa* infection. The incidence of *P. aeruginosa* colonization/infection would reduce by almost 50% if these measures have been done. Finally, routine admission screening and targeted environmental monitoring in specific wards seems to be efficiently, and the rate of water fitting contamination can be used as an index to place more emphasis on infection control procedures.

Conflicts of interest statement

None declared.

Funding sources

Hu Bijie received grant support from Special Fund for Health-scientific Research in the Public Interest: Research & application for the prevention & control of nosocomial infections coursed by multi-drug resistant bacteria (No. 201002021).

Acknowledgments

We gratefully thank the nurses and medical staff of two SICUs, who cooperated fully with this study. And we also thank Dr. Liang Qin and Dr. Lili Tao for their valuable suggestions and the review of manuscript.

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