Original Article

Emergence of Unusual Nonfermenting Gram-Negative Nosocomial Pathogens in a Saudi Hospital

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SUMMARY: This study aimed to determine the frequency of isolation and prevalence of drug resistance in nonfermenting Gram-negative bacilli (NFGNB) other than *Pseudomonas aeruginosa* and predisposing factors for the acquisition of nosocomial infections caused by these emerging pathogens in a Saudi tertiary care hospital. A total of 125 nonduplicating NFGNB nosocomial strains were isolated, of these, 68 (54.4%) were *Acinetobacter baumannii*, 26 (20.8%) *Stenotrophomonas maltophilia*, 14 (11.2%) *Alcaligenes faecalis*, 12 (9.6%) *Chryseobacterium indologenes*, and 5 (4%) *Ralstonia pickettii*. MICs of 11 antibiotics were determined using the reference broth microdilution method. With the exception of colistin that inhibited 100% of *A. baumannii* isolates, trimethoprim/sulfamethoxazole that inhibited 100% of *S. maltophilia* isolates, and carbapenems that inhibited 100% of *A. faecalis* isolates, none of the tested antimicrobial agents inhibited 100% of the other NFGNB spp. Our results emphasize that clinicians and microbiologists should consider *A. faecalis*, *C. indologenes*, and *R. pickettii* as emerging nosocomial pathogens. In addition, local resistance data are essential for helping physicians in deciding an appropriate antibiotic for empirical therapy of infections with these emerging and unusual NFGNB.

INTRODUCTION

Nonfermenting Gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic nonspore-forming bacilli. They occur as saprophytes in the environment and some are also found as commensals in the human gut (1,2).

NFGNB are ubiquitous in nature, particularly in soil and water, and are characterized by resistance to multiple antibiotics. In a hospital environment, NFGNB can be isolated from instruments and other fomites as well as the skin and hands of healthcare workers. Moreover, these pathogens often cause various nosocomial infections in seriously ill and immunocompromised patients, thereby resulting in high mortality rates (3–5).

The most commonly occurring NFGNB are *Pseudomonas* spp., *Acinetobacter* spp., *Stenotrophomonas maltophilia*, *Alcaligenes* spp., *Flavimonas*, *Oryzihabitants*, *Sphingobacterium*, *Burkholderia* spp., *Cepacia*, *Achromobacter* spp., *Bordetella* spp., *Camamonas* spp., *Methylobacterium* spp., *Oligella* spp., *Ralstonia* spp., *Psychrobacter* spp., *Roseomonas* spp., *Shewanella* spp., *Sphingobacterium* spp., and *Elizabethkingia* spp. (3,6).

Pseudomonas aeruginosa is the best known and most important clinically significant pathogen among NFGNB (7). Over the last few years, the pathogenicity and epidemiological features of *Acinetobacter baumannii* have been largely described in nosocomial infections (8–10); however, little information is available regard-

ing other NFGNB, many of which are considered as having minor clinical significance. Nevertheless, their involvement in human infections is likely underestimated, partly due to frequent misidentification by phenotypic methods commonly used in clinical laboratories (3,9). Hence, most studies dealing with antimicrobial susceptibility of NFGNB have focused on *P. aeruginosa* and *A. baumannii* spp., particularly the multidrug-resistant clinical isolates (7–9). To date, relatively few studies have explored the epidemiology and susceptibility profiles of unusual NFGNB (2–5,9–12). However, to the best of our knowledge, there are no such reports originating from Saudi Arabia.

The present study aimed to determine the frequency of isolation and prevalence of drug resistance in NFGNB (other than *P. aeruginosa*) and to identify predisposing factors for the acquisition of nosocomial infections caused by these emerging pathogens in a Saudi tertiary care hospital. Improved knowledge of these features could help the management and selection of empirical therapies for such infections.

MATERIALS AND METHODS

This study was conducted at King Khalid Hospital, a 350-bed tertiary care hospital in Najran, a city in southwestern Saudi Arabia, during a 6-month period from October 2012 to March 2013. Specimens of consecutive nonduplicating NFGNB strains, other than *P. aeruginosa*, were isolated from blood, urine, respiratory secretions (i.e., sputum, tracheal aspirate, and bronchoalveolar lavage), stool, pus, throat swabs, nasal swabs, and tips of central venous catheters from different patients (1 isolate/patient) who were hospitalized for ≥ 48 h and classified according to the Centers for Disease Control and Prevention/National Healthcare

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Safety Network (CDC/NHSN) criteria (13).

Medical and demographic data of the hospitalized patients were obtained using a questionnaire, which included age and gender, type of infection (urinary tract infection, respiratory tract infection, skin and soft tissue infection, and primary bacteremia), underlying disease (diabetes mellitus, chronic renal disease, chronic pulmonary disease, and malignancy), presence of intravascular or urinary tract catheters, history and length of stay in the intensive care unit (ICU), and histories of previous antibiotic therapy, recent hospitalization, and recent surgery.

All clinical samples were initially screened on blood agar, MacConkey agar, and TSI agar plates for isolation of NFGNB. The isolates were further identified to the species level using standard methods (3,14) and verified using the VITEK-2 (ID-GN card) system (bioMérieux, Marcy-l'Etoile, France) according to the manufacturer's instructions.

Antimicrobial susceptibility testing: MICs of 11 antibiotics were determined using the reference broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (15). The antibiotics tested included ceftazidime (CAZ), cefepime, piperacillin/tazobactam (TZP), imipenem

(IMP), meropenem (MEM), gentamicin (GEN), amikacin (AMK), ciprofloxacin (CIP), levofloxacin (LVX), trimethoprim/sulfamethoxazole (SXT), and colistin (CL). Quality control was performed using *Escherichia coli* ATCC 25921 and *P. aeruginosa* ATCC 27852.

Data were analyzed by WHONET 5.4 program. Numerical data were compared using the Student's *t*-test, and categorical data were compared using the chi-squared test or Fisher's exact test, where appropriate. All comparisons were 2-tailed, and *P* values < 0.05 were considered statistically significant.

RESULTS

A total of 125 nonduplicating NFGNB nosocomial strains, other than *Pseudomonas* spp., were isolated during the study period, of which 68 (54.4%) were *A. baumannii*, 26 (20.8%) *S. maltophilia*, 14 (11.2%) *Alcaligenes faecalis*, 12 (9.6%) *Chryseobacterium indologenes*, and 5 (4%) *Ralstonia pickettii*. The primary clinical specimens included tracheal aspirate (29.6%), sputum (25.6%), pus (22.4%), and urine (12%) (Table 1).

A comparison of the clinical characteristics of NFGNB-infected hospitalized patients showed no

Table 1. Distribution of all NFGNB among various clinical samples

Organism (no.)	Clinical sample, no. (%)					
	Urine	Sputum	Endotracheal tube	Pus	Blood	Body fluids
<i>A. baumannii</i> (68)	6 (8.8)	18 (26.5)	24 (35.3)	12 (17.6)	5 (7.4)	3 (4.4)
<i>S. maltophilia</i> (26)	4 (15.4)	6 (23.1)	8 (30.8)	5 (19.2)	3 (11.5)	0 (0)
<i>A. faecalis</i> (14)	2 (14.3)	4 (28.6)	0 (0)	7 (50)	0 (0)	1 (7.1)
<i>C. indologenes</i> (12)	3 (25)	2 (16.7)	3 (25)	3 (25)	0 (0)	1 (8.3)
<i>R. pickettii</i> (5)	0 (0)	2 (40)	2 (40)	1 (20)	0 (0)	0 (0)
Total	15 (12)	32 (25.6)	37 (29.6)	28 (22.4)	8 (6.4)	5 (4)

Table 2. Demographic data and clinical characteristics of 125 hospitalized patients infected by NFGNB

Variable	Hospitalized patients infected by					<i>P</i>
	<i>A. baumannii</i> (<i>n</i> = 68)	<i>S. maltophilia</i> (<i>n</i> = 26)	<i>A. faecalis</i> (<i>n</i> = 14)	<i>C. indologenes</i> (<i>n</i> = 12)	<i>R. pickettii</i> (<i>n</i> = 5)	
Median age in years (range)	60 (22–78)	46 (16–54)	52 (34–68)	64 (40–82)	35 (31–38)	<0.001
Male (%)	41 (60.3)	14 (53.8)	8 (57.1)	6 (50)	3 (60)	0.962
Type of infection						
UTI	7	4	2	2	0	0.830
RTI	44	14	5	7	4	0.267
SSI	12	5	7	3	1	0.125
Primary bacteremia	5	3	0	0	0	0.508
Underlying diseases						
Diabetes	26	10	5	5	2	0.995
Chronic renal disease	10	4	0	1	0	0.488
Chronic pulmonary disease	5	3	1	3	2	0.103
Malignancy	4	2	0	2	0	0.486
Median days of hospital stay (range)	12 (7–18)	5 (3–6)	7 (2–12)	8 (4–14)	4 (2–5)	<0.001
ICU admission (%)	42 (61.8)	8 (26.9)	4 (28.6)	5 (41.7)	2 (40)	0.024
Prior invasive procedure (%)	55 (80.9)	12 (46.2)	5 (35.7)	7 (58.3)	3 (60)	0.001
Prior surgery (%)	27 (39.7)	6 (23.1)	2 (14.3)	1 (8.3)	0 (0)	0.035
Prior antibiotic therapy (%)	28 (41.2)	10 (38.5)	6 (42.9)	5 (41.7)	2 (40)	0.986

UTI, urinary tract infection; RTI, respiratory tract infection; SSI, skin and soft tissue infection.

Table 3. Nonsusceptibility rates of NFGNB

Antimicrobial agent	% of isolates (intermediate/resistant)				
	<i>A. baumannii</i>	<i>S. maltophilia</i>	<i>A. faecalis</i>	<i>C. indologenes</i>	<i>R. pickettii</i>
TZP	0/32.4	0/84.6	0/14.3	16.7/33.3	0/20
CAZ	0/91.2	0/96.2	0/28.6	0/66.7	20/60
FEP	0/45.6	0/92.3	35.7/7.1	8.3/50	20/60
IMP	2.9/7.4	0/96.2	0/0	0/100	0/20
MEM	4.4/1.5	11.5/76.9	0/0	0/100	0/20
GEN	10.3/60.3	0/80.8	0/64.3	8.3/91.7	0/80
AMK	14.7/41.2	15.4/61.5	21.4/28.6	25/75	0/20
CIP	7.4/45.6	0/42.3	7.1/28.6	0/25	0/20
LVX	11.8/29.4	11.5/26.9	14.3/21.4	25/0	20/0
SXT	0/77.9	0/0	0/35.7	0/66.7	0/20
CL	0/0	0/26.9	0/35.7	0/58.3	0/20

TZP, piperacillin/tazobactam; CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; MEM, meropenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole; CL, colistin.

differences in gender; however, the mean age of patients infected with *C. indologenes* was higher than that of those infected with other pathogens (64.1 versus 34.8–58.5 years, $P < 0.001$). The mean hospital stay of patients infected with *A. baumannii* was longer than that of those infected with other pathogens (12.6 versus 4.7–8.5 days, $P < 0.001$). ICU admission because of *A. baumannii* ($n = 42$) was more common than that because of other pathogens ($P = 0.024$). Nosocomial infections caused by *A. baumannii* were more commonly associated with prior invasive procedures (81% versus 36–60%, $P = 0.001$) and surgery (40% versus 0–23%, $P = 0.035$) (Table 2).

The antimicrobials tested and the percentages of isolates determined to be intermediate and resistant are listed in Table 3. Overall, with the exception of CL that inhibited 100% of *A. baumannii* isolates, SXT that inhibited 100% of *S. maltophilia* isolates, and carbapenems (IMP and MEM) that inhibited 100% of *A. faecalis*, none of the tested antimicrobial agents inhibited 100% of the other NFGNB spp. The *A. baumannii* isolates were highly resistant to CAZ (91%), SXT (78%), GEN (71%), and AMK (56%). Notably, 12% and 7% of isolates were intermediately susceptible to LVX and CIP, respectively. More than 70% of the *S. maltophilia* isolates were resistant to cephalosporins, carbapenems, and aminoglycosides, whereas quinolone resistance rates were 38% and 42% for LVX and CIP, respectively. The *C. indologenes* isolates showed high resistance (50–100%) to most antibiotics, while quinolone resistance was 25% for CIP and LVX. On the other hand, *A. faecalis* and *R. pickettii* isolates showed low resistance (<50%) to most antibiotics.

DISCUSSION

NFGNB, other than *P. aeruginosa* and *A. baumannii*, are uncommon pathogens, and their clinical significance has not yet been fully established because these organisms are not often recovered from clinical specimens. In a previous study investigating 176 unusual NFGNB from Latin American medical centers during the first 6 years of the SENTRY antimicrobial surveillance program (1997–2002), *Burkholderia cepacia* was

the most frequently isolated pathogen (45.5%), followed by *Achromobacter xylosoxidans* (12.5%), *R. pickettii* (9.1%), *Alcaligenes* spp. (6.8%), and *Chryseobacterium* spp. (6.8%) (4). In the present study, *A. baumannii* and *S. maltophilia* accounted for 75% of all isolates, while 3 other genera (*Alcaligenes*, *Chryseobacterium*, and *Ralstonia*) accounted for the remaining 25%; however, these bacteria were often considered as culture contaminants or commensals. Our results showed that these bacteria were isolated from different infection types, thereby highlighting the need for clinicians and microbiologists to consider these unusual NFGNB as emerging nosocomial pathogens.

In this study, risk factors associated with unusual NFGNB nosocomial infections were advanced age of patients ($P < 0.001$), prolonged hospital stay ($P < 0.001$), ICU admission ($P = 0.024$), use of catheters or intubation ($P = 0.001$), and prior surgery ($P = 0.035$). Although part of these findings have already been described in previous studies of nosocomial infections caused by *A. baumannii* and *S. maltophilia* (11,16), our results extend these findings to other unusual NFGNB pathogens.

The resistance rates of *A. baumannii* to CAZ, aminoglycosides, and quinolones obtained in this study were higher than those reported in previous studies in Saudi Arabia (17,18). However, in a recent large-scale study investigating the resistance profiles of NFGNB from 5 regions of Saudi Arabia (19), the resistance rates of *A. baumannii* to CAZ, GEN, AMK, and CIP were 58–96%, 61–95%, 58–91%, and 50–81%, respectively. In this study, resistance rates of *A. baumannii* to carbapenem were almost comparable to those rates reported in a recent study in Saudi Arabia (19). In Europe, resistance rates of *A. baumannii* to IMP and MEM were reported 26% and 30%, respectively (20). Notably, some of our isolates were intermediately susceptible to LVX (12%) and CIP (7%), which suggest an increase in resistance to these drugs in the near future. CL and polymyxin B have emerged as alternatives against *A. baumannii*. In this study, all isolates were completely susceptible to CL. However, according to the SENTRY program, the resistance rate to polymyxin B ranged from 2.7% in Europe to 1.7% in North and Latin

America (20). The emergence of resistance to polymyxin will pose a serious therapeutic problem because no new antimicrobial agents are currently available for treatment of infections caused by this pathogen.

S. maltophilia is intrinsically resistant to most β -lactam agents, quinolones, and aminoglycosides; therefore, SXT is the drug of choice for the treatment of *S. maltophilia* infections (11). In total agreement with these findings, all *S. maltophilia* isolates in this study were completely susceptible to SXT. However, several reports have shown that the prevalence of strains that are resistant to SXT is increasing (10,21–23). In a recent study in Saudi Arabia (23), 2 cases of *S. maltophilia* infection were SXT-resistant. In contrast to other studies (24,25), our isolates showed sensitivity rates of >50% to CL, LVX, and CIP. Therefore, these agents can be used as alternative empiric therapy for *S. maltophilia* infection.

Antimicrobial susceptibility data on *Chryseobacterium* spp. remain very limited. Our isolates were resistant to most commonly used antibiotics but were sensitive to CL and quinolones. According to the results of the SENTRY program (4), the most active agents against *C. indologenes* were LVX (75% susceptibility), CIP (67%), and TZP (58%). Despite their low virulence, *Chryseobacterium* spp. are inherently resistant to many antimicrobial agents, which makes them potential agents of nosocomial infections (26).

The genera *Alcaligenes* and *Ralstonia* have been reported to be susceptible to SXT, CIP, and antipseudomonal β -lactams (3,4). Similar to these findings, $\geq 60\%$ of our isolates were susceptible to TZP, AMK, CL, and quinolones. However, delayed diagnosis and misinterpretation of the microbiological results—in the worst case scenario, disregarding a rare organism—can be hazardous to patients infected with these emerging pathogens (27).

In conclusion, accurate identification and susceptibility testing of NFGNB are critical to facilitate the understanding of the epidemiology of emerging pathogens. Our results highlighted that clinicians and microbiologists should consider *A. faecalis*, *C. indologenes*, and *R. pickettii* as emerging nosocomial pathogens. Moreover, local resistance data are essential for helping physicians choose an appropriate antibiotic for empirical therapy if an infection with any of these emerging unusual NFGNB is suspected.

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Conflict of interest None to declare.

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