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Original Article

Healthcare associated infections caused by non-fermenting Gram negative rods (NFGNR): six years of experience of a tertiary hospital

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Abstract

Recently non-fermenting Gram negative rods (NFGNR) are playing an important role in healthcare associated infections. This observational study in a tertiary care hospital of Dhaka city conducted during 01August 2007 to 30 June 2013 found that 34.8% isolated organisms from patients with healthcare associated infections were NFGNR. Majority (74.3 %) of these infections were occurring inside critical care areas. Pseudomonas and Acinetobacter together constituted 79.6% of the total NFGNR whereas Burkholderia cephacia complex (15.4%), Stenotrophomonas (4.3%) and Chryseobacterium species (0.7%) combined constituted remaining 20.4%. Out of total NFGNRs, Pseudomonas was responsible for highest number of catheter associated urinary tract infections (55.6%), ventilator associated pneumonia (46.3%), respiratory tract infection (65.8%) and surgical site infection (70.6%). Blood stream infection was predominantly caused by Burkholderia cephacia complex (33.5%) and Acinetobacter spp. (39.5%). Other than colistin most of the organisms were resistant to antibiotics commonly recommended for NFGNR.

Key words: NFGNR, HAI.

Introduction:

Non-fermenting Gram negative rods (NFGNR) are a diverse group of opportunistic pathogens that either do not utilize carbohydrate as a source of energy or utilize it in oxidative pathway[1]. It is difficult to manage patients infected by NFGNR because of failure to identify some of the members of this group in the laboratory and lack of options of treatment as most of them are resistant to common antibiotics [1-3]. Most members of this group remain in hospital environment as saprophyte and some in human gut as normal flora [4-5]. Previously many of them were considered as non virulent organisms. In recent time NFGNR have emerged as opportunist specially for immunosuppressed patients causing healthcare-associated infections (HAI) such as septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections (SSI) [4, 6].

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Important members of the group include Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia, and Burkholderia cephacia [3,7]. Most of them are frequently found resistant to common antibiotics including antipseudomonal penicillins and cephalosporins, aminoglycosides, tetracyclines, fluoroquinolones, trimethoprim-sulfamethoxazole, and carbapenems. However, polymyxins shows fairly consistent activity against multidrug-resistant strains of Pseudomonas, Acinetobacter and Stenotrophomonas species [3].

Among the NFGNR Pseudomonas is the most commonly isolated organism followed by Acinetobacter. Both are ubiquitous in nature and found in water and moist environment [2,8]. However, Acinetobacter is oxidase negative bacteria commonly found inside ventilators, humidifiers and catheters. The organism is increasingly responsible for outbreaks of infections among hospitalized patients, particularly in intensive care units. Antibiotic resistance has become a common phenomenon [9-11]. There are alarming reports of infections caused by carbapenem-resistant Acinetobacter spp [12-15].

Burkholderia cephacia complex (BCC), comprised of a group of some closely related bacterial species is another common non-fermentor that cause HAI and infections to patients suffering from cystic fibrosis [16]. As per recommendation by Clinical and Laboratory Standards Institute (CLSI) their susceptibility is limited to ceftazidime, meropenem, minocycline and cotrimoxazole only [17]. Stenotrophomonas maltophilia is another organism in this group though it is not considered as normal flora of human body. The organism is inherently resistant to anti-pseudomonal drugs and sensitive to co-trimoxazole. For detection of antibiotic susceptibility CLSI recommends broth dilution test, but if disc diffusion test is to be performed it should be done only against minocycline, levofloxacin and co-trimoxazole. Antimicrobial sensitivity of Chryseobacterium, another bacteria of hospital environment causing neonatal infections with high mortality rate, should be determined by dilution method as disc diffusion method does not give reliable results.

In Bangladesh there is hardly any comprehensive report on healthcare associated infections caused by these organisms. Lack of laboratory facilities to correctly identify some members of this group is an important factor for under reporting of these organisms. This study was undertaken to observe the role of NFGNR in causing healthcare associated infections in a tertiary care hospital of Dhaka city.

Materials And Methods:

This observational study on non-fermenting Gram negative rods was carried out in a tertiary care corporate hospital of Dhaka city. As a part of infection control program the hospital has got an ongoing active surveillance for detection of healthcare associated infections. For this study data were collected from 01 August 2007 to 30 June 2013 during which a total of 87608 patients were admitted in the hospital. Among them 1451 patients suffered from healthcare associated infections. Total number of non-fermenting Gram negative rods isolated from HAI cases during this period was 602 which constituted the study population. Determination of HAI was carried out by the surveillance protocol formulated by the hospital authority as follows. Every morning, temperature chart along with patient's identification (ID) number, collected from nurse stations, was sent to infection control (IC) team (who have received training on data collection). The team then entered the ID number of febrile patients (temperature >100°C) into the computer to retrieve patient's relevant information regarding infection. This included culture sensitivity test, complete blood count (CBC), use of clinical devices such as urinary catheter, respiratory equipments, central venous line etc, chest X-ray report and other pathological test reports indicating infection that occurred at least 48 hours after hospital admission. Suspected patients were visited by the IC team before including them as a case of HAI. If there was any confusion whether the patient was eligible to be included as HAI, treating physician was consulted. Isolation of organism/s from the appropriate sample of a febrile patient relevant with appropriate clinical features and/or positive pathological tests indicating infection was the criteria for inclusion as study subject.

Most of the common bacteria were identified by standard manual methods practiced in the hospital laboratory [18]. However, identification of non-fermenting Gram negative rods (NFGNR) which included Pseudomonas, Acinetobactor, Burkholderia, Stenotrophomonas and Chryseobacterium needed few more other tests [5,19]. The organisms that gave doubtful results were identified by automated identification system (Phoenix BD). Automated identification system was only used for those bacteria that gave confusing results and posed problems for identification. Antibiotic sensitivity was determined by disc diffusion method [20]. Selection of antibiotics for each group of bacteria were made as per recommendation made by Clinical and Laboratory Standards Institute (CLSI) 2010, guideline [17]. Pseudomonas aeruginosa ATCC 27853 was used as control strain. Antibiotics used for sensitivity test by disc diffusion method were amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, gentamycin, meropenem, co-trimoxazole, piperacillin, piperacillin + tazobactam and colistin.

Data collection was done by members of infection control team who filled up the HAI surveillance form for each patient. Data were analyzed by entering the data in SPSS version [12].

Results:

During August 2007 to June 2013, a total of 87608 patients were admitted, of which 1451 had healthcare associated infections (HAI) with an infection rate of 1.6 %. From these patients 1726 organisms were isolated, of which 602 (34.8%) were non-fermenting Gram negative rods that included Pseudomonas, Acinetobacter, Burkholderia Stenotrophomonas and Chryseobacterium species. Though age of the subjects ranged between 5 days to 103 years the mean and median were 51.2 and 57 years respectively. The age of 43% of total HAI affected patients were 60 plus and males predominated over females (62% vs. 38%).

Table 1 showed the distribution of 602 non-fermenting Gram negative rods (NFGNR). Pseudomonas and Acinetobacter

species together constituted more than three quarter (79.6%) of the total NFGNR whereas Burkholderia (15.4%), Stenotrophomonas (4.3%) and Chryseobacterium species (0.7%) together constituted less than a quarter (20.4%).

Table 2 showed that out of total NFGNR induced infections most of the urinary tract infections (UTI; 83.3%) were caused by Pseudomonas. Similarly Pseudomonas was responsible for highest number of catheter associated urinary tract infections (CAUTI; 55.6%), ventilator associated pneumonia (VAP; 46.3%), respiratory tract infection (RTI; 65.8%) and surgical site infection (SSI; 70.6%). However blood stream infections were predominantly caused by Burkholderia cephacia complex (BCC; 39.5%) and Acinetobacter species (33.5%). Only 4 Chryseobacterium species were isolated and all were found in blood stream infection.

Most (74.3%) of the organisms were isolated from the patients at critical care areas and remaining 25.7% were isolated from the patients admitted in non critical areas such as cabins and high density units (wards) (table 3). Critical care areas included general Intensive Care Unit (ICU), Neonatal ICU (NICU), Pediatric ICU (PICU), Cardiothoracic ICU (CTICU), Neuro-ICU and Coronary Care Unit (CCU) (table 3). Major contributor was the general ICU where more than half (307; 51%) of the NFGNRs were isolated. Pseudomonas predominated in ICU, PICU and non-critical areas whereas Acinetobacter predominated in NICU, CTICU and Neuro ICU.

Table 4 showed antimicrobial sensitivity of isolated NFGNRs. Among the Pseudomonas species highest (56.9%) sensitivity was recorded against the combination of piperacillin-tazobactam. This was followed by piperacillin (44.5%) and meropenem (40.6%). Almost two third of the Pseudomonas were resistant to remaining drugs. Only 23.9% and 20.1% of isolated Acinetobacter specimen were sensitive to meropenem and amikacin respectively. Sensitivity to other antibiotics was even lesser ranging between 4.1 to 18.7%. However, Burkholderia showed good sensitivity to ceftazidime (85.7%), meropenem (85.7%), piperacillin-tazobactam ((84.2%) and cotrimoxazole (78%). only 57% of stenotrophomonas were found sensitive to cotrimoxazole and 50% to ciprofloxacin. Though disc diffusion method is not recommended for Chryseobacterium, sensitivity to piperacillin-tazobactam and meropenem were found to be 33.3% and 25% respectively. Colistin was found sensitive to all of the isolated Pseudomonas, Acinetobacter, Stenotrophomonas and Chryseobacterium species. Burkholderia cephacia complex were resistant to colistin.

Table-1: Distributions of isolated non-fermenting gram negative rods (NFGNR)

NFGNR	No	%
Pseudomonas spp.	266	44.2
Acinetobacter spp.	213	35.4
Burkholderia cephacia complex	93	15.4
Stenotrophomonas spp.	26	4.3
Chryseobacterium spp.	4	0.7
Total	602	100

Table 2: Healthcare associated infections caused by NFGNR

NFGNR	UTI	CAUTI	VAP	RTI	BSI	SSI	Other	Total
Pse	10 (83.3)	30 (55.6)	124 (46.3)	25 (65.8)	36 (21.6)	36 (70.6)	5 (41.7)	266
Aci	1 (8.3)	21 (38.8)	107 (39.9)	10 (26.3)	56 (33.5)	13 (25.5)	5 (41.7)	213
Bur	0 (00)	2 (3.7)	20 (7.5)	2 (5.3)	66 (39.5)	2 (3.9)	1 (8.3)	93
Ste	1 (8.3)	1 (1.9)	17 (6.3)	1 (2.6)	5 (3.0)	0 (00)	1 (8.3)	26
Chr	0 (00)	0 (00)	0 (00)	0 (00)	4 (2.4)	0 (00)	0 (00)	4
Total	12 (100)	54 (100)	268 (100)	38 (100)	167 (100)	51 (100)	12 (100)	602

Note: 1. Pse= Pseudomonas; Aci= Acinetobacter; Bur= Burkholderia; Ste= Stenotrophomonas; Chr= Chryseobacterium; UTI= urinary tract infection; CAUTI= catheter associated urinarytract infection; VAP= ventilator associated pneumonia; RTI= respiratory tract infection (other than VAP); BSI= blood stream infection; SSI= surgical site infection. Note 2: Figure in the parenthesis is the percentage among each type of HAI

Table-3: NFGNR causing healthcare associated infections in critical and noncritical areas

NFGNB	Non-critical area	ICU	NICU	PICU	CTICU	CCU	Neuro ICU	Total
Ps	75 (48.4)	147 (47.9)	17 (26.6)	3 (30)	6 (27.3)	5 (50)	13 (38.2)	266
Aci	44 (28.4)	108 (35.2)	34 (53.1)	2 (20)	8 (36.4)	1 (10)	16 (47.1)	213
Bur	33 (21.3)	40 (13.0)	7 (10.9)	2 (20)	6 (27.3)	3 (30)	2 (5.9)	93
Sten	3 (1.9)	12 (3.9)	2 (3.1)	3 (30)	2 (9.0)	1 (10)	3 (8.8)	26
Chry	0 (00)	0 (00)	4 (6.3)	0 (00)	0 (00)	0 (00)	0 (00)	4
Total	155	307	64	10	22	10	34	602

Note: 1. Figures in parenthesis indicates percentage among each area; 2. Critical care areas include Intensive care unit (ICU), Neonatal ICU (NICU), Paediatric ICU (PICU), Cardiothoracic ICU (CTICU), Coronary care unit (CCU) and Neuro-ICU

Table-4: Antimicrobial sensitivity pattern of isolated NFGNB (showing susceptible percentage)

Antibiotics	Pseudomonas N= 266	Acinetobacter N= 213	Burkholderia N= 93	Stenotrophomonas N= 26	Chryseobacterium N= 4
Amikacin	34.5	20.1	6.7	00	00
Aztreonam	39.6	4.1	60	00	00
Cefepime	35.3	15.7	30.7	22.2	00
Ceftazidime	35	13.7	85.7	21.4	00
Ciprofloxacin	34.3	16.5	53	50	50
Gentamycin	30.8	17.4	4	14	00
Meropenem	40.6	23.9	85.7	00	25
Piperacillin	44.5	8.9	75	40	00
Piperacillin + tazobactam	56.9	16.9	84.2	44.4	33.3
Cotrimoxazole	-	18.7	78	57	-
Colistin	100	100	00	100	100

Discussion:

Recently there is a worldwide upsurge of healthcare associated infections caused by NFGNR. Management of infections caused by these organisms is difficult for two distinct reasons. First, it is difficult to identify them as most of the laboratories are not yet fully organized to do so. To some extent this is a universal problem as many of these organisms were previously classed under Pseudomonas or other genera and until recently they were considered as bacteria of lesser importance because of their relatively less virulent nature [21,22]. And the second difficulty is that, many of them are inherently resistant to common antibiotics [3-5].

Literatures reveal that among NFGNRs, Pseudomonas and Acinetobacter species play major role in causation of HAI, worldwide [8]. This study showed similar trend, as Pseudomonas and Acinetobacter together constituted almost eighty percent of our study population. This scenario is specially pronounced in intensive care units [8]. One of the important reasons for their dominance is that they have the ability to thrive in moist environment and have the capacity to survive in dry and inanimate surface even for up to six months [23-25]. Pseudomonas species is also inherently resistant to many antimicrobial agents. Susceptible strains are usually treated by piperacillin, ticarcillin, ceftazidime, cefepime, carbapenems, aminogylcosides or fluoroquinolones alone or in combinations. Recently there is alarming rise of multi drug resistant strains along with a few reports of pan resistance [26]. Our study also revealed a disheartening scenario. Other than colistin no antibiotic could confidently be singled out for empirical treatment. Only piperacillin-tazobactam combination showed sensitivity against more than half (57%) of isolated Pseudomonas. Sensitivity to other antipseudomonal antibiotics was poor (ranging between 30-44%). Enzyme production, over expression of efflux pumps, porin deficiencies, and target-site alterations could be the major mechanisms for drug resistance among Pseudomonas and other Gram-negative nonfermenters [6].

Acinetobacter is another organism responsible for various healthcare associated infections including bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection [27,28]. Drug resistance is very common and treatment option has become severely depleted. For treatment of drug resistant Acinetobacter, carbapenem and colistin are the agents of choice though strains resistant to all antimicrobial agents, including colistin have been reported [28]. This has created a serious threat for treatment of healthcare associated infections [29]. In our study we found that all were sensitive to colistin though only 23.9% were sensitive to carbapenem.

Burkholderia cephacia complex (BCC) one of the common pathogenic NFGNBs worldwide after Pseudomonas, Acinetobacter and Stenotrophomonas species has emerged as an important cause of morbidity and mortality in hospitalized patients largely because of high intrinsic antibiotic resistance[16]. BCC is responsible for many cases of septicemia and pneumonia in intensive care, oncology and hemodialysis units. In addition it can cause infection of skin and genitourinary tract as well [30,31]. In this study we found that 96% of isolated BCC were involved in bloodstream and respiratory tract infection with negligible infections at other sites.

A common mistake made by many laboratories is that BCC is reported as Pseudomonas. BCC is intrinsically resistant to anti-Pseudomonal agents like aminoglycosides, antipseudomonal penicillins and polymyxins which are commonly used against Pseudomonas infections. In such situations treatment failure is inevitable when aforementioned antibiotics are given to a BCC infected patient because of misdiagnosis by the laboratory. This highlights the importance of proper identification of NFGNR [16].

Chryseobacterium meningosepticum is an organism associated with meningitis in premature neonates [32]. They are found primarily in soil and water and even can survive in chlorine-treated water supplies. Colonization of patients through respirators, intubation tubes, humidifiers, incubators and syringes has been documented [33,34]. As an opportunistic pathogen they infect mainly newborns and immunocompromised hosts of all age groups causing meningitis, bacteremia, pneumonia, endocarditis, infections of skin, soft tissue, occular and other infections [35,36]. They are mostly resistant to aminoglycosides, tetracyclines, chloramphenicol, erythromycin, clindamycin, and teicoplanin. However, fluoroquinolones, minocycline, doxycycline, trimethoprim-sulfamethoxazole and rifampin can be used depending on susceptibility [35]. In this study total number of isolated Chryseobacterium were only 4 which preclude us to make any useful comment on antibiotic sensitivity. Moreover, we used disc diffusion method for detection of antimicrobial sensitivity which is not the recommended for Chryseobacterium.

Stenotrophomonas maltophilia was previously known as Pseudomonas maltophilia and also as Xanthomonas maltophilia. Maltophilia is the only species under this genus. S. maltophilia is associated with bacteremia, endocarditis, respiratory, urinary tract, CNS soft tissue, bone marrow and occular infections. Infections caused by S. maltophilia are particularly difficult to manage because they are frequently

resistant to many antimicrobial agents [37,38]. Though co-trimoxazole is the main therapeutic option, limited data suggest that ciprofloxacin, ceftazidime or ceftriaxone, and ticarcillin/clavulanate, alone or in combination with other antibiotics, may be considered as alternative options [39]. In our study only 57% of isolated Stenotrophomonas were sensitive to co-trimoxazole. Colistin is the only antibiotic with 100% susceptibility against isolated Pseudomonas, Acinetobacter, Stenotrophomonas and Chryseobacterium. Burkholderia cephacia is inherently resistant to colistin. But, problem with colistin is that it carries the risk of nephrotoxicity particularly among elders [3].

Literatures suggest that worldwide HAI caused by non-fermenting Gram negative rods are on rise. Our study also reveal similar scenario and it is a matter of concern that almost one out of three organisms causing HAI is NFGNR which are showing an alarming rate of antimicrobial resistance. We have hardly any data on antibiotic sensitivity pattern of NFGNR originated from the community. It is expected that resident organisms of hospital specially of ICU are relatively more resistant to antibiotics than those coming from community. Our hospital's annual antibiogram also shows that organisms of indoor patients are more resistant to antibiotics than that of outdoor patients which corroborates with findings of other literatures. This study is reflecting the situation prevailing in a tertiary care hospital of Dhaka city and it can be guessed that situation of other hospitals are not far different.

Unfortunately, with exception of a few, most of our laboratories are not yet organized enough to identify various NFGNR with confidence. Consequently, due to poor laboratory support the physicians are facing difficulties in treating infections caused by these organisms. Patients are unnecessarily being exposed to expensive antibiotics and prolonged hospital stay with increased morbidity and mortality. Lack of facilities as well as lack of awareness among the healthcare personnel is partly responsible for this situation. Identification of the NFGNR with appropriate sensitivity testing will play an important role in minimizing morbidity and mortality of patients suffering from healthcare associated infections.

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