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Demographic and microbiological profile of cystic fibrosis in Durban, South Africa

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Cystic fibrosis (CF) necessitates long-term treatment with multiple antibiotics creating selection pressure for the development of antibiotic resistance in infecting and/or colonizing organisms, impacting on disease management, morbidity and mortality. Sputum samples were obtained from patients attending the only two CF clinics in Durban over a year. The patient demographics and clinical data were recorded. Bacterial isolates were subjected to identification, susceptibility testing and phenotypic screening for extended spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo-β-lactamases (MBLs). Twenty-five patients constituted the study sample. The most common genotype was F508del and the most common pathogen was *Pseudomonas aeruginosa* with susceptibility to antibiotics ranging from 14-100% with marginal differences between mucoid and non-mucoid phenotypes. All *P. aeruginosa* isolates were putative ESBL producers and 75% were putative MBL producers. The incidence, prevalence and susceptibility patterns of bacterial pathogens and colonizers isolated from cystic fibrosis patients should be closely monitored to optimize management and treatment options in a disease requiring chronic antibiotic therapy which increases the propensity for the development of antibiotic resistance.

Key words: *Pseudomonas aeruginosa*, cystic fibrosis, extended spectrum β-lactamases (ESBLs), metallo-β-lactamases (MBLs).

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease caused by a mutation in the gene of the CF transmembrane regulator (CFTR) resulting in high morbidity and early mortality (Coutinho et al., 2008). In South Africa, approximately 1 in 20 individuals in the white population, 1 in 55 in the population of mixed-race and 1 in 90 black Africans carry a CFTR mutation (The South African Cystic Fibrosis Consensus Document, 2012). CFTR mutations vary considerably between populations and regions of the world with F508del constituting approximately 66% of all CF mutations globally (Saleheen and Frossard, 2008). The F508del mutation further accounts for up to 81% of all CF alleles in the South African white/caucasian population (Goldman et al., 2001), 53% in South Africans of mixed-race but is rarely detected in black African populations (Maseka et

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al., 2013).

The primary cause of long term complication and frequently death among CF patients is chronic bacterial infection of the respiratory tract with *Pseudomonas aeruginosa, Staphylococcus aureus* and *Haemophilis influenzae* as common causative bacteria (Hauser et al., 2011), although many other opportunistic bacteria have also been isolated, notably *Burkholderia cepacia* complex (BCC), *Stenotrophomonas maltophilia* and *Acinetobacter* spp., while, *Aspergillus* spp., non-tuberculosis mycobacteria and respiratory viruses have also been implicated (Saiman and Siegel, 2004). The most problematic bacterial infections are caused by *P. aeruginosa* and BCC and are characterized by significant reduction in lung function and low responsiveness to antibiotic therapy because of antibiotic resistance (Drevinek et al., 2008). Few disease states have high prevalence of antibiotic–resistant infections as does CF with 25-45% of adult CF patients estimated to be chronically infected with multi-resistant bacteria within their respiratory tracts (Cystic Fibrosis Foundation, 1994). Both bacteria also pose the risk of epidemic spread within the CF community, with the BCC being distributed among CF patients to much smaller extent (3 – 30%) as compared to *P. aeruginosa* (70 – 80%) (Drevinek et al., 2008). Neonates with CF have structurally normal lungs and no *P. aeruginosa*, but non-mucoid *P. aeruginosa* is acquired after variable time periods (LiPuma, 2010). The prevalence of *P. aeruginosa* increases with age and non mucoid *P. aeruginosa* is converted to mucoid *P. aeruginosa* which is usually associated with increasing lung deterioration with time (Govan and Deretic, 1996; Li et al., 2005).

This cross sectional observational study describes the demographic and microbiological profiles of CF in patients attending the only two dedicated CF clinics in the public and private health sectors in Durban, South Africa.

MATERIALS AND METHODS

Ethical considerations

This study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu Natal, South Africa (BE148/11).

Study sample

Patients attending the only two CF clinics (one public and the other private) in Durban, South Africa on scheduled clinic days over a 12 months period from June 2012-June 2013 formed the study sample and only 1 sputum sample (after expectoration) was sourced from each patient. The demographic and clinical data recorded included race, gender, age, age of first diagnosis and CFTR genotype (if known).

Microbiology

Twenty – five sputum samples were obtained (15 from the public sector and 10 from the private sector clinic). Bacterial and fungal isolates were identified and minimum inhibitory concentrations (MICs) for the bacterial isolates were determined using the VITEK MS and the VITEK 2 systems (bioMérieux, USA) with MICs analyzed according to CLSI guidelines (CLSI, 2012) for benzyl penicillin, oxacillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefuroxime, cefotaxime, cefoxitin, ceftazidime, cefepime, ertapenem, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, moxifloxicin, erythromycin, clindamycin, teicoplanin, vancomycin, tetracycline, tigecycline, fusidic acid, mupirocin, nitrofurantoin, colistin and trimethoprim/ sulfamethoxazole appropriately. *Streptococcus pneumoniae* ATCC 49619 and *Enterococcus faecalis* ATCC 29212 served as controls for Gram-positive identification and susceptibility respectively, *Shigella sonnei* ATCC 25931 and *Escherichia coli* ATCC 25922 served as controls for Gram-negative identification and susceptibility respectively and *Candida albicans* ATCC 14053 was the control for fungal identification.

All isolates were screened for extended-spectrum β-lactamase (ESBL) production using the double disc synergy method (Begum et al., 2013), AmpC β-lactamase production using the cefoxitin disc sensitivity test, inducible AmpC β-lactamase production using the disk antagonism test (Upadhay et al., 2010) and metallo-β-lactamase (MBL) production using the imipenem-EDTA combined disk test (Yong et al., 2002).

RESULTS

Twenty-five out of a total of 42 patients currently registered with the CF clinics constituted the study sample, representing 60% of the known patient cohort. There were 14 adults and 11 children, 14 males and 11 females, ranging in age from 2-33 years. Twenty-three (92%) of patients were white, 1 was of mixed-race and 1 was Indian. Of the 23 white patients, 18 were homo-zygous and 2 heterozygous for the F508del mutation, one showed the 3659 del C, 1 showed 1 copy each of the ∆1507 and 1 the E60X mutations and 1 was unknown. The CFTR genotypes of the other patients were unknown. Most of the patients were diagnosed in infancy or *in utero*. Only 1 patient was diagnosed in adulthood (Table 1).

Twenty-two bacterial and 6 fungal isolates were identified (Table 1). Patients harboured 3 different *Candida* spp. and 6 bacterial species other than the mucoid and non-mucoid *P. aeruginosa* in 12 different permutations. *P. aeruginosa* constituted the vast majority of bacterial isolates at 15 (68%).

The antibiotic susceptibility of *P. aeruginosa* isolates is shown in Table 2 with a marginal difference in susceptibility between the mucoid and non-mucoid phenotypes. *E. cloacae* was resistant to ampicillin, amoxicillin/clavulanate and cefoxitin; *K. pneumonia* was resistant to amoxicillin /clavulanate, cefoxitin and tobramycin; *B. cepacia* was resistant to meropenem and ciprofloxacin, *S. aureus* was resistant to benzyl penicillin and rifampicin and *Streptococcus mitis* showed intermediate resistance to erythromycin. All isolates were susceptible to colistin, used against multi-drug resistant isolates as a last resort because of its nephrotoxicity. All 20 (100%) Gram-negative isolates yielded a positive test for ESBLs and 15

Table 1. Patient demographics, age of CF diagnosis, CFTR genotype and microorganisms isolated from sputum.

Patient	Age	Gender	Race	Age of diagnosis	Genotype	Microorganisms Isolated from Sputum
1	2	M	W	Birth	Homozygous F508del	Normal respiratory tract bacterial flora
2	3	M	C	1 Year	Unknown	Normal respiratory tract bacterial flora; *Candida dubliniensis*
3	3	F	W	Birth	Homozygous F508del	Normal respiratory tract bacterial flora
4	4	F	W	1 Year	homozygous F508del	Normal respiratory tract bacterial flora; *C. dubliniensis*
5	5	F	W	Birth	homozygous F508del	Normal respiratory tract bacterial flora
6	7	M	W	3 months	homozygous F508del	*S. mitis S. maltophilia; Candida albicans*
7	8	F	W	13 Months	homozygous F508del	Normal respiratory tract bacterial flora
8	8	M	W	Birth	homozygous F508del	Normal respiratory tract bacterial flora
9	10	M	W	17 months	F508del /G551D	Mucoid *P. aeruginosa*
10	10	M	W	1 week	homozygous F508del	Non-mucoid *P. aeruginosa*
11	12	F	W	3 months	homozygous F508del	Non Mucoid *P. aeruginosa*
12	15	M	W	*In vitro*	Unknown	Mucoid and non-mucoid *P. aeruginosa; E. cloacae; C. albicans*
13	16	M	W	13 months	3659 del C	Mucoid and non-mucoid *P. aeruginosa*
14	18	F	W	22 months	homozygous F508del	*E. cloacae*
15	19	F	W	*In vitro*	homozygous F508del	Mucoid *P. aeruginosa; K. pneumonia; C. albicans*
16	20	M	W	Birth	Heterozygous F508del	Mucoid *P. aeruginosa*
17	21	F	W	9 months	homozygous F508del	Mucoid and non-mucoid *P. aeruginosa*
18	21	M	W	1 week	homozygous F508del	Normal respiratory tract bacterial flora
19	24	M	W	6 weeks	homozygous F508del	Normal respiratory tract bacterial flora; *C. albicans*
20	25	M	I	4 years	Unknown	*S. aureus*
21	25	F	W	1 month	∆1507; E60X (1 copy each)	*B. cepacia*
22	26	F	W	*In vitro*	homozygous F508del	Non-mucoid *P. aeruginosa*
23	28	M	W	17 months	homozygous F508del	Mucoid *P. aeruginosa*
24	32	F	W	20 years	homozygous F508del	Normal respiratory tract bacterial flora
25	33	M	W	1 year	homozygous F508del	Mucoid and non-mucoid *P. aeruginosa; Candida glabrata*

F- female; M- male; I- Indian; C- mixed race; W- white.

Table 2. Susceptibility of *P. aeruginosa* isolates to selected antibiotics.

Antibacterial agent	Susceptibility (%)	
	Mucoid *P. aeruginosa* (n= 8)	Non-mucoid *P. aeruginosa* (n= 7)
Piperacillin/-tazobactam	6 (75)	7 (100)
Ceftazidime	7 (88)	7 (100)
Cefepime	6 (75)	6 (86)
Imipenem	8 (100)	6 (86)
Meropenem	7 (88)	7 (100)
Amikacin	4 (50)	3 (43)
Gentamicin	2 (25)	1 (14)
Tobramycin	7 (88)	5 (71)
Ciprofloxacin	6 (75)	4 (57)
Colistin	8 (100)	7 (100)

(75%) were positive for MBLs. Although 18 (90%) of isolates screened were positive for AmpC β-lactamase production on the basis of resistance to cefoxitin on the disc sensitivity test, none were inducible according to the

Table 3. Results of phenotypic screening for β-lactamases.

β-lactamase	*P. aeruginosa* (n=15)	*E. cloacae* (n=2)	*K. pneumonia* (n=1)	*B. cepacia* (n=1)	*S. maltophilia* (n=1)
ESBL	15	2	1	1	1
AmpC	13	2	1	1	1
Inducible AmpC	0	0	0	0	0
MBL	12	1	0	1	1

disk antagonism test (Table 3).

DISCUSSION

Although CF occurs in all South African population groups, it is better described in the white and mixed race populations while its prevalence in the black population is less well known (Westwood et al., 2006) indicating potential under-diagnosis as the black population group comprises greater than 80% of the total KwaZulu Natal population. Notwithstanding the fact that CF patients may be managed outside of the two CF clinics, possible under-diagnosis may be attributed to CF being omitted from differential diagnoses in this population group, poor access to medical care and misdiagnosed as malnutrition, indicative of CF, is very common in the black population for poverty-related reasons (Maseka et al., 2013). Further, just two of the 13 CF clinics in South Africa are located on the coast in Durban and may not be easily accessible to people from the inner and rural areas. The predominant CFTR genotype was F508del as recorded previously for the South African population (Maseka et al., 2013).

Many organisms that are isolated from sputa of CF patients are pathogens (e.g. *S .aureus*) that often progress to colonize the upper respiratory tract or are common environmental organisms that behave as opportunistic pathogens (e.g. *P. aeruginosa*) (Valenza et al., 2008; Cardoso et al., 2008). *S. aureus* is usually the first pathogen to infect and colonize the airways of CF patients (Hauser et al., 2011), while *P. aeruginosa* occurs in early childhood with prevalence increasing with age such that as many as 80% of patients with CF are infected with *P. aeruginosa* by the time they reach the age of 20 (Li et al., 2005). The median age recorded in this study was 16 explaining the predominant isolation of *P. aeruginosa*.

Patients with CF are at risk of multi-resistant infections as a result of endo-bronchial bacterial infections that in most cases cannot be eradicated (Aaron, 2007) and frequent high dose antibiotic therapy is an essential part of CF management. Patients are exposed to multiple courses of antibiotics both chronically and intermittently, and this introduces selective pressure for the development of antibiotic resistance in infecting and/or colonizing organisms (The South African Cystic Fibrosis Consensus Document, 2007).

In comparison, non-mucoid *P. aeruginosa* showed lesser susceptibility to imipenem, ciprofloxacin and the aminoglycosides while mucoid *P. aeruginosa* were less susceptible to meropenem, the cephalosporins and the piperacillin-tazobactam inhibitor combination. Although differences in antimicrobial susceptibility between mucoid and non-mucoid *P. aeruginosa* have been documented in many studies, the significance is yet to be ascertained. Notwithstanding the marginal differences in susceptibility observed in this study, it is postulated that the exo-polysaccharide/alginate compromises access to antibiotics such that the mucoid isolates are exposed to sub-inhibitory concentrations of antibiotics facilitating the evolution of resistance (Hauser et al., 2011).

All *P. aeruginosa* isolates in this study were putative ESBL producers and were resistant to most of cephalosporin generations. Infections with ESBL-producing pathogens occur in patients who have recently received broad spectrum antibiotics, particularly third-generation cephalosporins and quinolones as is the case with chronic therapy in CF. Multi-drug resistance to the aminoglycoside, fluoroquinolone and β-lactam antibiotic classes was also evident and attributed to the co-carriage of resistance genes on the same genetic determinants of resistance, whether plasmids, transposons or integrons, severely limiting treatment options (Kanj and Kanafan, 2011).

The incidence, prevalence and susceptibility patterns of different microorganisms in the sputa of CF patients should be closely monitored to optimize management and treatment options in a disease requiring chronic antibiotic therapy to reduce morbidity and mortality. The complexity and diversity of β-lactamase expression in *P. aeruginosa* from CF patients, necessitates early detection to inform efficacious antibiotic therapy as antibiotic options are limited not only in the treatment of CF but in the treatment of all infections globally.

Conflict of Interests

The authors have not declared any conflict of interests.

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