Emerging cystic fibrosis pathogens: incidence and antimicrobial resistance

Annarita Magni[1], Alessandra Giordano[1], Carlo Mancini[1], Claudia Pecoraro[2], Paola Varesi[1], Serena Quattrucci[2], Maria Trancassini[1]

[1]Department of Public health Sciences, "La Sapienza" University of Rome, Italy
[2]Cystic Fibrosis Center, Department of Pediatrics, Azienda Policlinico Umberto I, Rome, Italy

SUMMARY

We examined the frequency of isolation and the antimicrobial resistance of *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* in cystic fibrosis patients from 2000 to 2004. Strains susceptibility to tobramycin, piperacillin/tazobactam, imipenem, gentamicin, ciprofloxacin and ceftazidime was determined by disc diffusion assay. *B.cepacia* complex showed a very high resistance also to ciprofloxacin reaching 100% in 2004. *S.maltophilia* and *A.xylosoxidans* showed high rates of antimicrobial resistance both aminoglycoside and ciprofloxacin. It is very important to monitor the percentage of isolation of these species over time to verify strains resistance to antibiotics and also to test new combinations of antimicrobial agents.

KEY WORDS: Cystic fibrosis, Bacteria isolation, Antibicrobial resistence

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Chronic pulmonary disease and concomitant airway inflammation are the major causes of morbidity and mortality in CF patients due to non fermentative bacteria like *Pseudomonas aeruginosa* (Chmiel *et al.,* 2002; Lyczak *et al.,* 2002). The microbiology of the CF lung is not static, and the dynamic ability of bacteria to adapt to new environments would indicate that the health of CF patients is constantly under threat from emerging pathogens (Beringer & Appleman, 2000; Steinkamp *et al.,* 2005) such as *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* (Graff & Burns 2002; Tan *et al.,* 2002). It may be the result of the use of more aggressive antibiotic chemotherapies directed against *P.aeruginosa* (Talmaciu *et al.,* 2000). While the impact of these potentially emerging pathogens on morbidity and mortality remains under study, there are limited therapeutic options available due to intrinsic and acquired resistance to antimicrobial agents (Gibson *et al.,* 2003).

Our study examined the frequency of microbiological isolation and antimicrobial resistance to quinolones, beta-lactams and aminoglycoside in the years 2000-2004 of *B.cepacia* complex, *S.maltophilia* and *A.xylosoxidans* in CF patients attending the Cystic Fibrosis Center of the Department of Pediatrics at "La Sapienza" University of Rome.

Expectorated sputum and tracheal aspirate samples were collected from 420 patients (mean age 17 years) every three months, or more often during acute respiratory infections for a total of 8531 specimens. Specimens spread (10µl) on common culture media and, for the isolation of *B.cepacia* complex, on a selective media Oxidative-Fermentative Base, Polymyxin B, Bacitracin and Lactose (OFPBL) agar in 2000-01 and after on *B.cepacia* Selective Agar (BCSA) were incubated at 35°C for up to 5 days to allow

Corresponding author
Dr. Maria Trancassini
Dipartimento di Scienze di Sanità Pubblica,
Università "La Sapienza" di Roma
Via Aldo Moro, 5 - 00185 Roma
E-mail: maria.trancassini@uniroma1.it

slow-growing bacteria to develop. All *B.cepacia* complex, *S.maltophilia* and *A.xylosoxidans* strains were identified by the API 20NE system (Bio-Merieux). Susceptibility to antimicrobial agents was determined by the disc diffusion assay on Mueller Hinton II Agar (Becton Dickinson) with commercial antimicrobial susceptibility discs (Becton-Dickinson) according to standard for *P.aeruginosa* of the National Committee for Clinical Laboratory Standards 2000 and 2004 (NCCLS 2000, NCCLS 2004). The antibiotics tested were: tobramycin (TOB), piperacillin/tazobactam (TZP), imipenem (IPM), gentamcin (GM), ciprofloxacin (CIP) and ceftazidime (CAZ).

The significance of the data was determined by χ^2 test.

Table 1 shows the percentage incidence of the three bacterial species studied. *S.maltophilia* and *B.cepacia* complex however varied during the period from 2000 to 2004. During 2001, an increase in *S.maltophilia* was observed with respect to the previous year from 3.2% to 4.5%, and continued to increase reaching a maximal value in 2003 (5.8%). Throughout 2002 the frequency of isolation percentages of *B.cepacia* complex increased from 3.2% in 2001 to 5.9% in 2002, while it decreased to 2.6% in 2004. In contrast, the frequency of isolation of *A.xylosoxidans* remained relatively constant, showing values around 3%. Table 2 shows the percentages of resistance of the three bacterial species from 2000 to 2004. As the intrinsic resistance of *S.maltophilia* to monobactams and *B.cepacia* complex to aminoglycosides are well-known, *S.maltophilia* was not tested for resistance to IPM and the latter bacteria were not evaluated for resistance to TOB or GM. It is apparent that *S.maltophilia* is the most resistant microorganism, showing the highest percentage of resistance to both aminoglycosides and CIP, wherein the frequency of resistance increased from 34.0% in 2000 to 60.2% in 2004 ($p<0.01$). An increase in resistance to CAZ was also observed from 2000 to 2004 (43.0% vs. 52.3%, respectively). *B.cepacia* complex showed an increase in resistance to IPM from 2001 to 2004 (57.0% vs. 84.0%, respectively ($p<0.05$). A similar trend was observed for resistance to CIP, which reached 100% in 2004. The frequency of resistance to TZP and CAZ was relatively constant, averaging 35.5% for the former and 26.0% for the latter. *A.xylosoxidans* showed high resistance to TOB, GM, and CIP. Resistance to CIP, as already observed for *S.maltophilia* and *B.cepacia* complex, increased from 45.0% in 2000 to 83.6% in 2004 ($p<0.001$). A lower frequency of resistance to TZP ($p<0.05$), IPM ($p<0.01$), and CAZ ($p<0.01$) was observed, even though significant increases were observed from 2000 to 2004. Many children receive elective intravenous antibiotic treatment every three months but this intensive use of antibiotics theoretically increases the likehood of opportunistic infection with inherently resistant organisms, such as *S.maltophilia*, *B.cepacia* complex and *A.xylosoxidans* (Steinkamp *et al.,* 2005). While *B.cepacia* complex has emerged as a major pathogen in CF, there is little published data on the extent of *A.xylosoxidans*. and *S.maltophilia* pathogenic role in CF patients. Recently available data on American CF patients show that the overall prevalence of *S.maltophilia* was 8.4% and that there were significant differences among CF care centers; *A.xylosoxidans* is about 4.4% (Cystic Fibrosis Foundation. Patient registry, 2002). Our

TABLE 1

Year	*S.maltophilia*	*B.cepacia complex*	*A.xylosoxidans*
2000 (1644)[a]	3.2 (53)[b]	3.4 (56)[b]	3.4 (55)[b]
2001 (1662)[a]	4.5 (74)[b]	3.2 (53)[b]	3.7 (61)[b]
2002 (1666)[a]	4.6 (77)[b]	5.9 (98)[b]	2.9 (48)[b]
2003 (1597)[a]	5.8 (94)[b]	4.4 (71)[b]	3.3 (53)[b]
2004 (1962)[a]	4.7 (93)[b]	2.6 (51)[b]	2.8 (55)[b]

[a]Specimens; [b]Strains number

TABLE 2

	2000	*2001*	*2002*	*2003*	*2004*
S.maltophilia					
TOB	75.0	73.2	78.0	74.5	78.5
TZP	55.1	49.3	58.7	66.3	53.8
GM	66.2	70.4	72.1	70.8	78.5
CIP	34.0	34.1	45.3	55.4	60.2
CAZ	43.0	39.3	49.1	53.3	52.3
B.cepacia complex					
TZP	35.2	32.1	36.0	37.1	37.3
IPM	57.0	72.4	76.3	74.9	84.3
CIP	80.5	94.1	94.2	98.6	100.0
CAZ	28.3	25.0	26.1	24.9	26.1
A.xylosoxidans					
TOB	80.0	76.5	81.0	90.6	94.5
TZP	4.1	14.5	13.0	14.5	16.4
IPM	7.0	12.3	13.0	26.4	32.7
GM	87.3	80.7	87.0	83.2	89.1
CIP	45.0	67.5	69.1	81.1	83.6
CAZ	22.0	41.6	44.2	43.4	47.3

data confirmed the increase in *B.cepacia* complex and *S.maltophilia* isolation mainly during last year. For *B.cepacia* complex the increase was due probably to use of new selective media BCSA. These selective media showed a very good sensitivity and specificity in the recovery and provided a first screen for the presence of *B.cepacia*-like organisms in cultures (Henry *et al.,* 1999). The decrease of isolation from 2003 could be due to the segregation techniques adopted by the CF centre.

The rise in incidence for *S.maltophilia* may be the result of the use of more aggressive antibiotic chemotherapies directed against *P.aeruginosa* also in our CF center as reported during the last decade in many CF centers (Denton & Kerr, 1998; Demko *et al.,* 1998). However its clinical relevance and implications in pulmonary deterioration remain unclear (Goss *et al.* 2002).

The relevance of *A.xylosoxidans* in CF lung disease is poorly understood. We found this bacterium in a significant proportion of patients with no apparent effect on their clinical course. However, it is rather difficult to distinguish colonization from infection, because this bacteria was found only intermittently in the majority of our CF patients. To conclude, although the exact pathogenetic roles of *S.maltophilia* and *A.xylosoxidans* on CF patients has not yet been established, the percentage of isolation should be monitored over time and above all their resistance to the different class of antibiotics verified. As far as *B.cepacia* complex is concerned, the antimicrobial management in CF patients remains a complex problem and hence some form of synergic testing to examine and prevent antagonism should form a basis to help guide more efficient combinations of agents used.

Finally, antibiotic selection should be based on periodic isolation and identification of pathogens and review of the antimicrobial susceptibility profile following the latest CLSI guide especially for *B.cepacia* and *S.maltophilia* (CLSI 2006).

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