135 *Pseudomonas aeruginosa* (PA) resistant to colistin (CL) in Italian cystic fibrosis (CF) patients

G. Manno[1], P. Morelli[1], M. Mentasti[1], R. Casciaro[1], N. Cirilli[2], R. Gagliardini[2], A. D'Aprile[3], F. Gioffrè[4], D. Scuteri[4], G. Melioli[1]. [1]*G.Gaslini Institute, Genova, Italy;* [2]*CF Centre, Ancona, Italy;* [3]*CF Centre, Cerignola, Italy;* [4]*CF Centre, Soverato, Italy*

Background: CL has emerged as a relevant therapeutic option for the treatment of PA pulmonary infection in CF. The development of resistance to this agent under selective pressure is not unexpected, even if it may occur at lower extend. Objective: Monitoring the susceptibility to CL of a collection of PA from Italian CF pts.
Methods: 295 PA strains (151 non-mucoid, 113 mucoid and 31 SCV) from 178 pts from 4 Italian CF Centres, were tested by CLSI disk diffusion method for 12 antipseudomonal drugs and CL using a 10 mcg disk. Isolates with resistance to CL disk were re-tested by Etest in MH agar, after at least 10 repeated sub-culture in blood agar. The genetic relatedness of all isolates was determined by BOX-PCR.
Results: Four PA isolates (1 mucoid, 1 non-mucoid and 2 SCV) from 4 patients showed resistance to CL by disk diffusion and confirmed by Etest. The resistance level was high, with The MIC ranging from 12 to 32 mcg/ml. The 2 SCV strains were multi-drug-resistant, while the mucoid isolate was susceptible to the other anti-pseudomonal drugs tested. The CL-resistant PA isolates showed different genetic profiles by BOX-PCR. 3/4 patients chronically colonized by CL-resistant PA had several previous antibiotic treatment with aerosolized CL.
Conclusions: This is the first description of PA CL-resistance in Italian CF patients. Although in very low percentage (2.2%) this emergence need concern in the extensive and prolonged use of this important therapeutic option. Moreover, in the CF microbiology workout, seems to be mandatory the CL (or polimixin B) routinely testing in all PA isolates.
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136 Evolution of antibiotic resistance in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients: results from a longitudinal study

S. Campana[1], S. Tekle[1], N. Ravenni[1], C. Braggion[1], G. Taccetti[1]. [1]*Cystic Fibrosis Center, University of Florence, Florence, Italy*

Pulmonary infection is the main cause of morbidity and mortality in cystic fibrosis (CF) patients. Patients who are subject to *P. aeruginosa* are treated with parenteral combination antibiotic therapy on a routine basis (three times per year) and although this approach has notably improved the quality of life and longevity of CF patients, it favors the selection of highly resistant strains. We evaluated the percentage of resistance to commonly used antimicrobials against *P. aeruginosa* isolates from 200 CF patients referred to the Tuscan Regional Cystic Fibrosis Center between 1996–2006. The chemosensitivity profile was created by including *P. aeruginosa* strains isolated every 6 months from each CF patient, for a total of 1715 strains. The table illustrates the prevalence of resistance to the principal antimicrobials used during the observation period. These data demonstrate a dramatic increase in the percentage of *P. aeruginosa* strains resistant to the main classes of antimicrobial agents. This increased resistance of *P. aeruginosa* strains emphasizes the urgent need to optimize therapeutic protocols for the treatment of infections due to this important opportunistic pathogen in order to limit the selection and occurrence of highly resistant strains to the currently used antimicrobial agents.

Percentage of resistant *P. aeruginosa* strains from 1996–2006

Antibiotic	Percentage of resistant strains[a]										
	1996 (93)	1997 (93)	1998 (108)	1999 (102)	2000 (150)	2001 (157)	2002 (163)	2003 (220)	2004 (213)	2005 (216)	2006 (200)
MEP	–	–	16	17	13	10	23	22	21	21	23
IMP	14	22	25	24	23	15	25	22	24	26	24.5
CAZ	3	19	20	16	19	7	21	18	14	17	19
TIC	8	14	27	24	25	18	23	27	24	27	53
NN	11	14	19	7	13	11	23	36	26	32	27
CIP	16	14	24	12	23	16	37	32	34	33	36.5

[a]Total numbers of strains are listed in parentheses after the year.

137 The sensibility of *Ps. aeruginosa* isolates in children with cystic fibrosis

E. Chioroglo[1], S. Sciuca[1], O. Turcu[1]. [1]*Pediatry, State Medical and Pharmaceutical University, Chisinau, Moldova*

Aim: to study the sensibility of *Ps.aeruginosa* isolates in children with cystic fibrosis (CF).
Materials and Methods: study included 13 children with CF at the age of 2–18 year with chronic *Ps. aeruginosa* infection. 43 probes of sputum were evaluated microbiologically in exacerbation of bonhopulmonary disease. Antibiogram was appreciated by method of diffusion in Muller-Hinton agar with antibiotics from standard disks. Diagnosis was confirmed in all children based on the clinical data, positive sweat test and/or positive molecular diagnostic. ΔF508 mutation was revealed in 10 children, 1 child – G542X/N1303K mutation, 2 cases – unidentified mutation. Bronchopulmonary system affecting was characterized by bronchiectasis in children (61.5%), infectious bronchiolitis, pulmonary fibrosis, chronic bronchitis – in all children.
Results: *Ps. aeruginosa* was determined in all children with chronic infection in diagnostic concentration. Study showed high sensibility to imipenem – 95.8%, fluoroquinolones (ciprofloxacin – 92.6%), cephalosporin's II–III generation (cefoperazon – 96.3%, ceftazidim – 83.8%, ceftriaxone – 66.6%); aminoglycosides (tobramycin – 93.5%, gentamicin – 74.4%), penicillines antipseudomonas also presented marked sensibility to piperacillin – 86.3%, piperacillin/tazobactam – 95%. There was an evident resistance of examined isolates to ampicillin – 100%, amoxicillin – 100%, doxycycline – 100%, cefazolin – 86.7%, cefuroxime – 90.8%, cefotaxime – 54.3%, macrolides (clarithromycin – 100%, azithromycin – 95.5%).
Conclusions: Chronic airways colonization with *Ps. aeruginosa* in children with CF manifests high sensibility in vitro to ciprofloxacin, cefoperazon, ceftazidime and antipseudomonas penicillines, tobramycin, which could be recommended in the therapeutic programs of *Ps. aeruginosa* infection.

138 The effect of incubation of sub-minimum inhibitory concentration of antibiotics on strains of *Pseudomonas aeruginosa* (PA) in a desiccation survival model

I. Clifton[1], M. Denton[2], F. M'Zali[2], D. Peckham[1]. [1]*Regional Adult CF Unit, Leeds, United Kingdom;* [2]*Department of Microbiology, Leeds General Infirmary, Leeds, United Kingdom*

Introduction: Prior to modern genetic typing methods cross-infection of PA between patients with cystic fibrosis (CF) was felt to be rare. Recently a number of studies have been undertaken that demonstrate the presence of clonal strains of PA infecting patients with CF. The aim of this study was to determine whether clonal strains of PA demonstrated differences in resistance to desiccation, and whether pre-incubation with sub-minimum inhibitory concentrations (MICs) of antibiotics affected desiccation resistance.
Methods: Strains of PA pre-incubated with or without antibiotics were studied in a dessication survival assay with controlled conditions of temperature and humidity. The survival curves were modelled to a Weibull curve using least squares error method using GraphPad Prism. Differences between curves were determined using the F-Test. $P < 0.05$ was taken to be significant.
Results: Pre-incubation with antibiotics did not have a consistent effect on the strains of PA. All of the non-clonal strains and two of the clonal strains demonstrated significant reduction in desiccation resistance following pre-incubation with tobramycin. Meropenem significantly reduced desiccation resistance in most of the strains and ceftazidime had the least effect on the strains studied.
Conclusions: Promoting bacterial survival through antibiotic exposure could have important clinical consequences by potentiating the risk of cross infection between patients with CF. Further studies are needed to investigate the impact of sub-MIC concentrations of different antibiotics have on promoting or inhibiting patients with CF.