BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2016
VOL. 73, NO. 2, 87–89
http://dx.doi.org/10.1080/09674845.2016.1165408

BIOMEDICAL SCIENCE IN BRIEF

Antimicrobial resistance in cystic fibrosis isolates of *Haemophilus influenzae*

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ARTICLE HISTORY Received 6 January 2016; Accepted 5 February 2016

KEYWORDS Antibiotic resistance; cystic fibrosis; haemophilus influenza

Cystic fibrosis (CF) is a life-shortening genetic condition, occurring in approximately 1 in 2-3000 live births, that compromises pulmonary function.[1] Patients with CF are prone to recurrent bacterial respiratory infections [1] and non-typable *Haemophilus influenzae* (NTHi) is commonly isolated from the sputa of children with CF.[2,3] The disease has no cure and patients typically undergo frequent antibiotic therapy to manage their condition.[4] There is good evidence that CF-related antibiotic therapy causes increased antibiotic resistance in organisms that infect CF patients, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*,[5,6] but less evidence that this increased resistance extends to NTHi. A previous longitudinal study of NTHi isolates from 30 CF patients over a 7-year period showed increased ciprofloxacin, cotrimoxazole and β-lactamase negative ampicillin resistance (BLNAR) compared to matched non-CF isolates.[7]

In the current study, CF and non-CF NTHi isolates were screened for resistance to a number of relevant antibiotics to determine if the CF isolates were more frequently resistant. Sixty-six consecutive presumptively identified NTHi isolates from routine diagnostic respiratory specimens of CF patients were collected during the period of January–December 2011 by the laboratories of the Prince of Wales Hospital (Sydney, NSW, Australia) and the Prince Charles Hospital (Brisbane, QLD, Australia). Eighty-six NTHi isolates were collected from routine diagnostic respiratory specimens of non-CF patients during the period of March–June 2012 by the laboratories of Royal Hobart Hospital, Hobart Pathology, Launceston General Hospital, Launceston Pathology and North West Pathology, Tasmania, Australia. Only non-identifiable data (specimen type, age and sex) were recorded.

Isolates were initially identified by colonial morphology on chocolate agar and X+V dependency using standard methods, and subsequently underwent PCR for the *fucK*, *sodC* and *hpd#3* species marker genes, using a previously described algorithm and methods.[8] Isolates were identified as NTHi if they were X and V growth factor dependent, *fucK* and/or *hpd#3* positive and *sodC* negative. We examined β-lactam, fluoroquinolone, macrolide and co-trimoxazole resistance because of the relevance of these antibiotics in managing respiratory infections [9] and chose different methods for detecting resistance to maximise sensitivity of detection. Resistance to β-lactam antibiotics such as amoxicillin is either attributable to β-lactamase production, which can be reliably detected by nitrocephin hydrolysis, or in BLNAR strains, to altered penicillin binding protein 3 (PBP3) which is usually associated with an asparagine to lysine amino acid substitution at position 526 (N526K).[9] Isolates were tested for β-lactamase presence using nitrocefin discs (Becton Dickinson, NSW, Australia), according to the manufacturer's instructions, and for BLNAR genotype using a previously described PCR method [10] that is more sensitive than minimum inhibitory concentration (MIC)-based methods.[9,11] MICs for azithromycin were determined using *E*-test (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions and using European Committee on Antimicrobial Susceptibility Testing (EUCAST) susceptibility testing media and interpretive criteria (R > 4 mg/L).[12] Co-trimoxazole susceptibility was determined using EUCAST disc diffusion methodology and interpretive criteria (R > 1 mg/L).[12] A two-step process was used for the detection of quinolone resistance. Isolates initially underwent a previously described naladixic acid disc diffusion screen [13] to detect isolates potentially carrying mutations in various quinolone resistance determining regions. Screen positive isolates underwent MIC determination for ciprofloxacin using *E*-test according to the manufacturer's instructions and using EUCAST susceptibility testing media and interpretive criteria (R > 0.5 mg/L).[12]

Differences in prevalence of β-lactamase activity, presence of BLNAR genotype, co-trimoxazole resistance and number of isolates with at least one resistance were analysed using a χ^2 test of independence. The difference in prevalence of azithromycin resistance was analysed using a Fischer's exact test because of the absence of

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Table 1. Antimicrobial susceptibility comparison between the CF isolates and non-CF isolates.

	CF isolates	non-CF isolates	*p*
Total No. of isolates	59	86	–
BLP (nitrocefin)	9 (15%)	22 (26%)	0.138
BLNAR (N526K PCR)	17 (29%)	15 (17%)	0.106
SXT-R (disc diffusion)	11 (19%)	12 (14%)	0.449
CI-R (*E*-test MIC)	0	0	–
AZ-R (*E*-test MIC)	0	4 (5%)	0.146
Total R	38 (64%)	47 (55%)	0.243

Notes. BLP = No. of positive nitrocefin results, BLNAR = No. of isolates negative for N526K PCR, SXT-R = No. of co-trimoxazole-resistant isolates by disc diffusion, CI-R = No. of ciprofloxacin-resistant isolates by *E*-test MIC, AZ-R = No. of azithromycin-resistant isolates by *E*-test MIC, Total R = No. of isolates with at least 1 of the above resistance mechanisms.

resistance in the CF group. All analyses were performed using Epi Info™ 7 (Centres for Disease Control and Prevention, Atlanta, USA) and a significant cut-off of $p < 0.05$.

The median age of the CF patients was 5 years (range = 0–51 years, IQR = 20), compared to 65 years (range = 0–92 years, IQR = 20) for the non-CF patients and the difference was statistically significant ($p < 0.001$) with a Welch Two Sample *t*-test using R version 3.2.0 (The R Foundation for Statistical Computing). All isolates were X and V growth factor dependant but seven CF isolates were excluded from the study as non-NTHi based on the *fucK/hpd#3/sodC* results.

The results for resistance are summarised in Table 1, but the overall finding is that no statistically significant difference in resistance between the CF and non-CF isolates was detected. This is in contrast to the previous study by Roman et al. [7] which reported significant differences between CF and non-CF isolates. A number of differences between that study and our study deserve further mention. The previous study involved a larger number of isolates ($n = 188$ CF and 188 non-CF) taken from a smaller cohort of CF patients ($n = 30$) but was a longitudinal study over a 7-year period; by contrast, our number of isolates was smaller but almost all isolates were derived from individual patients with a few repeat specimens and thus represents point prevalence rather than an accumulation of resistance in isolates from individual patients over time.

Our results of 21% β-lactamase positive strains (CF and non-CF combined) are similar to the 22% of Roman et al. and our 22% BLNAR strains compared to 3% can be explained by our use of a genotypic criteria (N526K) compared to the MIC-based method which is significantly less sensitive (12). However, we failed to find any strains (CF and non-CF combined) with reduced ciprofloxacin susceptibility (MIC > 0.5 mg/L) compared to 0/188 and 40/188 (21%) in non-CF and CF strains, respectively, by Roman et al. (MIC > 1 mg/L). Similarly, we found 19% and 14% cotrimoxazole resistance (CF and non-CF) using a breakpoint of >1 mg/L compared to 65 and 36% by Roman et al. using a breakpoint of >2 mg/L. Thus, for ciprofloxacin and cotrimoxazole where Roman et al. found a significant difference in resistance between CF and non-CF isolates, they also had much higher levels of overall resistance in their isolates (CF and non-CF) than we found, and this may be a reflection of different antibiotic use in Spain and Australia. In conclusion, our study shows that NTHi respiratory isolates derived from CF patients do not appear to be more resistant to antibiotics commonly used to treat respiratory infections than similar isolates from non-CF patients.

This report is an advance in biomedical science because NTHi is a significant pathogen in young CF patients and advice on the prevalence of resistance should be evidence-based and may be influenced by the prescribing practices of a particular region.

Disclosure statement

There are no conflicts of interest to declare.

Funding

This project was funded by a grant from the Clifford Craig Medical Research Trust (project 99, UTAS T0019623).

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