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Risk factors for respiratory tract bacterial colonization in adults with neuromuscular or neurological disorders and chronic tracheostomy.

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Short title: tracheal colonization of chronic tracheostomised adults

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Introduction

Adults suffering from neuromuscular or neurological disorders may need chronic tracheostomy as a consequence of chronic respiratory failure. Long term tracheostomy facilitates access to ventilation and allows the removal of bronchial secretions by aspiration to relieve the subject from bronchial obstruction.

These invasive procedures and the proximity of skin lead to persistent bacterial colonization of the respiratory tract in most chronically tracheostomised subjects. The diagnosis of pneumonia is complex in this population due to the difficulty and challenges in the identification of the causal bacteria. The incidence of pneumonia in chronic tracheostomised subjects is however higher than in non-tracheostomised subjects [1].

Several studies have documented the bacterial species colonizing the superior or lower respiratory tract in populations of chronic tracheostomised subjects both hospitalised or not. Their results showed a persistent colonization with a predominance of certain species but varied depending on the populations and the studies [2-9]. The best studied disease is chronic obstructive pulmonary disease (COPD). For instance, Mobbs *et al.* evaluated the oropharyngeal aerobic Gram-negative bacilli carriage at 6.6% in healthy individuals and at 29.7% in severe COPD [10, 11].

No large-scaled study of this type has focused on chronically tracheostomised subjects performed for a neurological or a neuromuscular disorder. This population is, however, often exposed to antibiotics, frequently carrier of multidrug-resistant organisms (MDRO) [12-14], susceptible to secondary swallowing disorders and at high risk of respiratory or lung super-infection.

The aim of this study is to describe the physiological respiratory bacterial flora and antibiotic resistance of tracheostomised subjects with a neurological or a neuromuscular

underlying disorder, and to identify the risk factors for respiratory colonization with potentially pathogenic bacteria in the absence of an active pneumonia.

Methods

Inclusion criteria

This prospective observational single center study was conducted from February 2015 to December 2016 in the ventilation unit of our hospital. This tertiary teaching hospital is a center of expertise in neurological impairment and manages a 10-beds ventilation unit specifically for the care of patient living with ventilatory machines at home.

The inclusion criteria were an age greater than 18, a tracheostomy for more than 6 months for a neuromuscular or neurological underlying disorder, admission in the dedicated unit in Raymond Poincaré hospital for less than 48 hours for an annual planned respiratory check-up. All patients or their legal guardian signed an informed consent form.

Exclusion criteria included active respiratory tract infection (fever $>38.4^{\circ}\text{C}$, worsening dyspnea or increase of aspirates and chest infiltrates on radiography), antimicrobial therapy or hospitalization within the previous 30 days.

For each subject included, data was collected in a standardized questionnaire including characterization of subjects, type of neurological or neuromuscular disorder, characterization of ventilation.

Medical history and characterization of ventilation were reported by the patients or their family. Tracheal aspirates from included subjects were sampled during the 48 first hours of hospitalization.

Bacteriological analysis of tracheal aspirate

Tracheal aspirates (TA) were obtained from the mid-part of the trachea with sterile catheters. In the laboratory, serial dilution of liquefied TA in sterile saline solution (1/1000 and 1/100000) were cultured on the following media: blood agar (aerobic and anaerobic),

chocolate agar in 5% CO₂ and Drigalski agar to select Gram-negative bacteria (Biomérieux, Marcy-l'Etoile, France) [15]. Bacterial identification was performed by Maldi-TOF mass spectrometry (Microflex and biotyper Bruker Daltonique®, Wissembourg, France). Bacteria were quantified and antibiotics susceptibility assayed only for potentially pathogenic bacteria: non-fermenting Gram-negative bacilli, *Enterobacteriaceae*, *Staphylococcus aureus*, *Haemophilus sp.*, *Moraxella sp.* and *Streptococcus pneumoniae*. Susceptibilities were determined by the disc diffusion method and interpreted according to the 2015 EUCAST/CASFM recommendations (<http://www.sfm-microbiologie.org>). For each patient, overall susceptibility for all the bacteria isolated from each TA was determined according to natural and acquired resistances, identifying antibiotics that would have been effective if the patient required to be treated.

Bacteriological analysis of humidifiers

Humidifiers of 7 adults included in the study were analyzed for bacteriological contamination by filtration on nitrocellulose membrane. Filters were cultured on Plate Count Agar and bacterial growth identify by mass spectrometry.

Statistical analysis

Descriptive statistics were presented as counts and percentage for qualitative data. Quantitative data were described by mean and standard deviation or median with interquartile. Groups were compared with chi-square test for qualitative data, and student test for quantitative data. Odds Ratio (OR) were estimated using logistic regression. Multivariate analysis was performed with a multivariate logistic regression including factors that had a p-value <0.15 in the univariate analysis. A p value of 0.05 or less was considered as significance.

Statistical analyses were performed using the R software 3.2.3 version.

Ethics committee

This study was reviewed and approved by the institutional review board CPP Ile-de-France XI and registered under ClinicalTrials.gov NCT02400086.

Results

Characteristics of included subjects

Of the 1128 admissions in this unit during this period, 264 were related to 131 tracheostomised patients, 77 of which were included. The remaining 54 patients were excluded because they did not meet inclusion criteria or could not be screened for inclusion.

Clinical and epidemiological characteristics of included patients were summarized in Table I.

Thirty-one subjects had received an antibiotic treatment within 6 months, twelve of them with Amoxicillin+Clavulanic-acid, and 40% of the treatment lasted more than seven days. A history of respiratory infections was frequent (62% of the subjects, n=48/77) with an occurrence within the previous year for 24 patients (31%).

Twenty-four patients were hospitalized within the past year for a mean duration of 16 days, half of them for respiratory issues.

Ventilation requirement characteristics

Collected data on ventilation practices are shown in Table I.

Exchange filters and heated humidifiers were common and 41 subjects (53%) received heated humidification at night and heat and moisture exchanger during the day.

Tracheal aspirate colonization

Sixty-nine of the 77 TA cultures were positive with at least one potentially pathogenic bacterium (90%) (Table II), among them 82.6% (n=57/77) were superior to 10^5 CFU/ml (74% of TA). Eighty two percent (n=63/77) of TA had bacterial load greater than the threshold of 10^5 CFU/ml which defining infection according to the French Microbiology Society (SFM)

(15). Mixed flora e.i; Gram negative and positive bacteria, was found for 71.8% of cases (n=56/77). Four TA remained sterile after 48 hours incubation and 4 TA had a non-pathologic oropharyngeal flora.

A total of 268 bacteria were isolated (3 to 4 per TA) with 168 potentially pathogenic bacteria (63%) including 120 Gram-negative bacilli (71%). The three species most frequently isolated were: *Staphylococcus aureus*, in 44% of TA (n=34/77); *Pseudomonas aeruginosa*, in 42% of TA (n=32/77); *Serratia marcescens*, in 38% of TA (n=29/77). Other *Enterobacteriaceae* were isolated in 25% of TA (n=19/77). Potentially pathogenic hydric bacteria (including *P. aeruginosa*, *S. marcescens*, *Stenotrophomonas maltophilia*) were found in 61% of TA (n=47/77). Both *P. aeruginosa* and *S. aureus* were found for 12 patients and both *S. marcescens* and *S. aureus* for 14 patients. Seventeen patients were colonized with *P. aeruginosa* and *S. marcescens* and 6 patients with the three species.

Moreover, on the 77 TA, 44 samples had more than 25 neutrophils per coding field (57%).

Bacterial resistance

Analysis of global resistance for each TA showed that Amoxicillin+Clavulanic-acid was active in 28% of cases, Cefoxitin in 32% and Cefotaxim in 35% of cases due to natural resistance of the three most isolated species. Conversely, Piperacillin+Tazobactam would have been effective in 75% of cases, Cefepime in 83%, imipenem in 81% and levofloxacin in 86% of cases. In five cases, use of a combination would have been required to be effective against all of the pathogenic micro-organisms identified.

The proportion of MDRO among the isolates was low: only one Ceftazidime resistant *P. aeruginosa* (n=1/32, 3%), one extended spectrum beta-lactamase (ESBL) producing *Citrobacter koseri* (n=1/48, 2%) and two Meticillin resistant *S. aureus* (MRSA) (n=2/34, 6%) were observed.

Humidifiers contamination

No species from TA analysed for the same patient on the same day was detected in humidifiers. Moreover, investigation of humidifiers revealed a contamination with environmental bacteria such as *Pseudomonas mendocina*, *Pseudomonas oleovorans*, *Sphingomonas paucimobilis* or *Kocuria palustris* but no potentially pathogenic bacteria, regardless of the type of water used.

Risk factors of tracheal aspirate colonization

TA were separated in different groups to statistically determine risk factors (the presence or absence of hydric bacteria, presence or absence of non-hydric pathogenic bacteria, presence or absence of *P. aeruginosa*, presence or absence of *S. aureus* and the amount of potentially pathogenic bacteria). Risks factors were analysed for all groups but only the results for presence or absence of *P. aeruginosa* and *S. aureus* are shown in Table III. Colonization of respiratory tract with *P. aeruginosa* was associated with living in a medical-care home ($p=0.01$, OR=3.8 [1.1; 15.1]) and the use of cuffed tracheostomy tube ($p=0.003$, OR=4.4 [1.1; 20.6]), as was the colonization with more than two potentially pathogenic bacteria and with hydric bacteria (data not shown) However, colonization with *S. aureus* was not associated with any factor related to the lifestyle or to the ventilation characteristics. Subjects lived in different medical-care homes without links between them. Of the 22 subjects with cuffed tracheostomy tubes, only 5 lived in medical-care homes.

Other modes of ventilatory support did not seem to have an impact on respiratory tract colonization. Type of disease, duration of tracheostomy, malnutrition, previous antibiotic treatment, history of hospitalization or respiratory infection, swallowing disorder and gastrostomy were not associated with colonization of the respiratory tract by any type of potentially pathogenic bacteria (data not shown).

Discussion

This study is the first one relating to colonization of respiratory tract in long-term tracheostomised subjects with neuromuscular or neurological disorders. The findings in the cohort of 77 subjects reflect the usual bacterial flora colonizing the respiratory tract in this population. Unlike other studies where chronic tracheostomised subjects had an average duration of tracheostomy of 1 to 2 years, subjects included in our study were tracheostomised for a much longer period, often longer than 13 years.

Potentially pathogenic bacteria were present in 90% of TA from chronic tracheostomised subjects for neuromuscular or neurological disorder in the absence of any infectious episode immediately prior to or at inclusion on our study. Three species were most frequently encountered, *P. aeruginosa*, *S. aureus* and *S. marcescens* but none predominates over the others. Lusuardi *et al.* described colonization of tracheal aspirates (TA) with 38% of *Pseudomonas aeruginosa*, 26% of *Serratia marcescens* and 7% of *Staphylococcus aureus* in 39 long-term tracheostomised non hospitalized patients with COPD or restrictive pulmonary disease. [3, 8]. Harlid *et al.* described TA with 72% of *S. aureus*, 72% of Gram positive bacilli and 46% of *Enterobacteriaceae* and 80% of the patients stayed colonized with the same species for 44 weeks on average [5].

Moreover, none of patients included has an active pneumonia (exclusion criteria). Our study highlights that TA is not the only contributing criterion in the diagnosis of acute infection in this population, when the usual thresholds are not specific for respiratory tract infection diagnosis. A diagnosis can be postulated but not confirmed by TA bacteriological analysis. In this setting there is little value in identifying infections with quantitative bacteriologic studies or cell counts of TA as was also observed by Lusuardi *et al.* [3] and Bartlett *et al.* [7].

The efficacy of antibiotics usually used to treat community-acquired respiratory infections e.i; Amoxicillin+Clavulanic-acid or Cefotaxime is limited by the high prevalence of *P. aeruginosa*, *S. marcescens* and other hydric bacteria, expressing chromosomal cephalosporinases and impermeability. Conversely, susceptibility for

Piperacillin+Tazobactam, Cefepime, Imipenem or Levofloxacin is frequent and are all options as empiric treatment of respiratory infection for these subjects despite their ability to select resistant bacteria. MDRO are uncommon in these subjects even if more than a third received antibiotics treatment within the last 6 months and those subjects infected were often in contact with a hospital environment. However, in the United-States, McCaleb *et al* detected an important proportion of MRSA in their long-term tracheostomised paediatric population [17] and Suda *et al* found a higher frequency of MRSA, ESBL-producing *Enterobacteriaceae* and Carbapeneme-resistant *Enterobacteriaceae* a patient population with spinal cord injuries than in the general population [12]. The high rate of acquired resistance was not revealed in our population: this discrepancy could be explained by the high rate of subjects living at home in the specific impaired population studied.

Pathogenic bacteria from the water predominate in TA suggesting contamination during care. In France, the National Agency for Drug Security (ANSM), recommends a change of water in humidifiers every day, the cleaning of humidifiers each week and preferably the use of sterile water, distilled water or boiled water [16]. In our study, the type of water used in heated humidifiers does not appear to be correlated with the colonization even of hydric bacteria. Environmental bacteria were isolated from humidifiers regardless the grade of water used.

As in long-term tracheostomised children, the presence of *S. aureus* in TA is frequent [17]. No risk factor for *S. aureus* colonization was identified among all compared items. The manual handling of the tracheostomy tube insertion site may be the source of this carriage.

Ventilation practices and techniques vary from one subject to another with few consequences on TA except the use of cuffed tracheostomy tube that is significantly associated with *P. aeruginosa* colonization of the respiratory tract. A survey conducted by Park *et al* in Korea in amyotrophic lateral sclerosis patients with long-term use of tracheostomy tube showed that almost all the patients and caregivers had an erroneous idea

about cuff volume and pressure [18]. *P. aeruginosa* colonization may be due to retention of secretions up to the cuff even though in our study, most of the cuffed tracheostomy tubes are deflated. Single use of this type of tracheostomy tube and weekly replacement routinely performed in France seems appropriate.

Potentially pathogenic bacteria such as *P. aeruginosa* are more frequently isolated in adults living in a medical-care home rather than at their own home, suggesting a higher pressure of selection or colonization. The more frequent exposure to water contaminated with *P. aeruginosa* in medical-care homes than in a normal home setting could be related to more complex or less well-maintained water networks.

This study has several limitations. First it is a single-center study with a limited sample size (n=77). However, the sample size is larger than most similar studies on this topic. Another point is that this cohort has included adults only; meanwhile the published studies also include children who might have different flora and type of infection, partly because they are not exposed to the same drugs and antibiotic pressure.

Secondly, we did not study the impact of antimicrobial resistance on specific outcomes in these patients. It would have been insightful to compare these outcomes to the use of specific antibiotics and compare the success rate to the percentage of resistance described in our cohort.

Finally, this study is an instant view of respiratory tract bacterial colonization in this population in absence of active pneumonia with the future goal of verifying the contribution of TA in the initiation of a probabilistic antibiotherapy in case of pneumonia for example, or to evaluate if certain ventilation practices can be a risk factor for pneumonia. A preliminary follow-up in medical records of 80% of this cohort shows a prevalence of pneumonia of about 20% during the year following the TA without identifiable risks factors. Most of the bacteria isolated by protected distal sampling during the acute pneumonia were similar to those found

in TA during the past year (data not shown). These initial results need to be explored and validated through further prospective surveys with a follow-up clearly defined.

In conclusion, this overview of the ventilation practices and techniques in long-term tracheostomised subjects with neuromuscular or neurological disorders reveals their diversity and the little influence of these ventilation practices on respiratory tract bacterial colonization. Pathogenic bacteria are a common finding in cultures of TA from this population in the absence of infection. Among the three predominant bacteria isolated, *P. aeruginosa* and *S. marcescens* and *S. aureus*, two are innately resistant to Amoxicillin+Clavulanic-acid that prevent the use of this common antibiotic in empiric treatment of lower respiratory tract infection in these subjects.

Conflict of interest

Declarations of interest: none

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Table I. General data and ventilation data of patients included in the study

Clinical and epidemiological characteristics	
Median age	36 years [min 20 – max 72]
Sex ratio	2.9 M/F
Median duration of tracheostomy	13 years [min 6 months – max 54 y]
Type of disease	- Duchenne muscular dystrophy: 33/77 (43%) - Others muscular dystrophies: 22/77 (29%)
Living place	- Home: 55/77 (71%) - Medical-care home: 22/77 (29%)
Malnutrition	23/77 (30%)
Immunosuppression	8/77 (10%)
Swallowing disorder	24/77 (31%)
Ventilation	76/77 (99%)
Ventilation 24/24h	58/76 (76%)
Ventilation during meal	63/76 (83%)
Exchange filters	57/76 (75%)
Heated humidifiers	57/76 (75%)
Type of water in humidifiers	- Bottle water: 21/57 (37%) - Sterile water: 18/57 (32%) - Tap water: 12/57 (21%)
Tracheostomy tube	- Cuffed: 18/77 (23%) with 10/18 (56%) deflated - Fenestrated: 10/77 (13%)

Table II. Proportion of Patients with specific Microorganism on TA Culture (n=77)

Organism	n (%)
<i>Staphylococcus aureus</i>	34 (44%)
<i>Pseudomonas aeruginosa</i>	32 (42%)
<i>Serratia marcescens</i>	29 (38%)
Other enterobacteria	27 (35%)
<i>Stenotrophomonas maltophilia</i>	11 (14%)
<i>Haemophilus sp</i>	11 (14%)
Beta-hemolytic <i>Streptococcus</i>	11 (14%)
<i>Streptococcus pneumoniae</i>	8 (10%)
<i>Acinetobacter baumannii</i>	6 (8%)
<i>Moraxella catarrhalis</i>	5 (6%)

Table III. Risk factors for colonization of TA

		<i>P. aeruginosa</i>				<i>S. aureus</i>		
		Presence (n=32)	Absence (n=45)	Univariate OR	Multivariate OR	Presence (n=34)	Absence (n= 43)	Univariate OR
Residence	Home	18 (32.7%)	37(67.3%)	1		23 (67.6%)	32 (74.4%)	
	Medical-care home	14 (63.6%)	8 (36.4%)	3.6 [1.3 ; 10.5]	3.8 [1.1 ; 15.1]	11 (32.4%)	11 (25.6%)	ns
Antibiotic last 6 month		11/30 (44.4%)	20 (36.7%)			12/32 (35.3%)	19 (44.2%)	
Tracheostomy duration median [IQ]		13.2 [6.8; 13.6]	14.4 [7.2 ; 22.6]	ns		16.6 [7.7; 22.3]	13.1 [6.5; 17.6]	
Ventilation during meal		26 (81.3%)	36 (80%)	ns		28 (82.4%)	34 (79.1%)	ns
Heated humidifiers		24 (75%)	33 (73.3%)	ns		23 (67.6%)	34 (79.1%)	ns
Exchange filters		26 (81.3%)	31 (68.9%)	ns		26 (76.5%)	32 (72.1%)	ns
Sterile water used in humidifiers		12 (37.8%)	9 (20%)	ns		8 (34.8%)	13 (38.2%)	ns
Fenestrated tracheostomy tube		4 (12.5%)	6 (13.3%)	ns		6 (17.6%)	4 (9.3%)	ns
Cuffed tracheostomy tube		13 (40.6%)	5 (11.1%)	5.5 [1.8 ; 19.2]	4.4 [1.1, 20.6]	6 (17.6%)	12 (27.9%)	ns
Time of change of tracheostomy tube :								
Less than once a week		10 (32.3%)	7 (15.6%)	ns		7 (21.2%)	10 (23.3%)	ns
Once a week or more		21 (67.7%)	38 (84.4%)			26 (78.8%)	33 (76.7%)	
Number of tracheal aspirate per day :								
Less than 5		14 (43.7%)	20 (44.4%)	ns		17 (50%)	17 (40.5%)	ns
5 to 10		10 (31.3%)	18 (40%)			11 (32.4%)	17 (40.5%)	
More than 10		8 (25%)	6 (13.3%)			6 (17.6%)	8 (19.0%)	

ns : not significant