Resistance Profile From Staphylococcus Aureus And Pseudomonas Aeruginosa Obtained From Tracheostomized Children

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Abstract

Background The tracheostomized patients exhibit high risks of bacterial infections, because the tracheal tube acts as a gateway to these microorganisms. The objective was to characterize microbiologically the tracheal secretion of tracheostomized children, to evaluate the biofilm formation, and to study the phenotypic and molecular profile of antimicrobial resistance of Staphylococcus aureus and Pseudomonas aeruginosa isolated. Methods The study collected 88 tracheal secretion samples. The material processed by phenotypic tests were performed for bacterial identification. For identification of the biofilm, the Congo red agar test and the plaque microtiter test were used, and the qPCR method was used to resistance verification. Results Were obtained 27 samples of S. aureus and 71 of P. aeruginosa . All S. aureus samples were positive for biofilm formation on Congo red agar test. In antibiogram test, S. aureus showed resistance to seven drugs. Regarding the identification of resistance genes, were amplificated bla Z in 42.8% from S. aureus and mec A in 28.6% of them. Pseudomonas aeruginosa presented resistance to eight drugs. The most frequent chromosomal genes were bla OXA with 66.7% and bla KPC with 58.3%. To plasmidial DNA, was highlighted bla NDM with 58.3% positive. Conclusion The S. aureus and P. aeruginosa characterization of colonization from lower respiratory tract associated with the use of the device in tracheotomy patients, and the physiology and antimicrobial resistance profile, will help health professionals to choose the most appropriate treatment to be administered in children with tracheotomy, increasing the chances of airway restoration and decannulation.

Introduction

Tracheostomy consists of a surgical opening in the anterior tracheal wall, into which a tracheal cannula is inserted. It's indicated in respiratory obstruction cases, subglottic stenosis, tracheomalacia and craniofacial syndromes, as well as providing access to the lower respiratory tract in cases as neurological and neuromuscular diseases[1].

Due the lack of protection as filtration, humidification and air heating, performed by the upper respiratory tract, tracheostomized patients exhibit high risks of virus and bacterial infections, because the tracheal tube acts as a gateway to these microorganisms[2, 3, 4]. The contamination and the consequent lower respiratory tract colonization are facilitated, and the main bacteria found were *Pseudomonas aeruginosa* and *Staphylococcus aureus*[5, 6]. Both microorganisms exhibit high resistance rates to β-lactams, the most commonly group used in medical routine[7].

In addition, bacteria that are frequently found in tracheostomy tubes are biofilm-forming, such as *S. aureus* often associated with lower respiratory tract infections[1, 3, 8, 9,10].

Besides, others resistance factors dissemination can be attributed to the genetic mutational processes and genetic material exchange, because of selective environmental pressure and multiresistant clone's proliferation[10]. Through is possible the plasmids transport resistant genes, that codes antibacterial information's, becoming the bacteria multiresistant. The mechanisms that become bacteria resistant are

decreased permeability of both wall and cytoplasmic membrane; target site antibiotics alteration; antibiotic expulsion by efflux pumps and β-lactamases enzymes production that inactivate the antibiotic. These mechanisms come from mutational processes and/or genetic material exchange[11, 12].

The indiscriminate antimicrobials use can lead to individual's normal microbiota alteration, which increases the risks of acquisition and colonization of resistant bacteria, as may select resistant microorganisms that exist in the airways. This may origins an increase in costs to the patient because of increased length of stay in hospitals, besides the prevention and treatment difficult[13].

To prevent possible respiratory infections, tracheostomized patients should exchange tracheostomy tube once a month, but the non-availability of the cannula by the Brazilian Public Health System, contributes to increase of change time, because patients have to pay for the device and the most of time don't have financial resources to do it. The aim of this research was to study the physiology and antimicrobial resistant profile by phenotypic and molecular methods of *P. aeruginosa* and *S. aureus* obtained from tracheostomized children tracheal secretion.

Methods

The research project was approved by the Ethics Committee of the Clinical Hospital of the Federal University of Goiás (CEP/HC/UFG), by number 32091014.6.1001.5078. Were isolated 27 *S. aureus* samples and 71 *P. aeruginosa* samples, obtained from tracheotomized pediatric patients' tracheal secretion. The samples were obtained from a terciary universitary hospital in Goiania's city.

Eighty-eight tracheal secretion samples were collected from 11 tracheostomized children, with age between zero months to eighteen years old, that using the tracheostomy cannula to ventilatory support and were not infected or with symptoms like fever, increased discharge, productive cough or any other unusual symptoms. Samples were collected prior to cannula removal and were taken twice each season, during the time of cannula replacement, which was performed between 30 and 45 days, according to the protocol.

The samples were collected from May 2017 to October 2018, the first being performed in autumn and the others in the following order: winter, spring and summer.

To collect of the secretion, the contents were aspirated with a syringe containing physiological solution, coupled to a probe, which was introduced into the tracheostoma, and the physiological solution was injected into it. The aspirated contents were deposited in a sterile vial and immediately sent for processing in the laboratory.

Microbiological analysis

Samples were sown in salty mannitol, MacConkey, chocolate and blood culture media. Cultures were incubated for 24 hours at 37 °C under aerobic conditions. After this period, the morphocolonial and

morphotintorial characterization was performed, as well as biochemical tests to identify isolated colonies, according to Procop and collaborators[14] and the ANVISA manual[15].

Biofilm production was analyzed according to FREEMAN et al[16] and Tendolkar et al.[17], with adaptations.

Antibiogram test

The *P. aeruginosa* and *S. aureus* samples were subjected to the antibiogram test using the Kirby and Bauer method[18]. To *P. aeruginosa*, were used discs of Ceftazidime (CAZ) 30µg, Imipenem (IPM) 10 µg, Gentamicin (GEN) 10 µg, Ciprofloxacin (CIP) 5 µg, Piperacillin and Tazobactam associated (PPT) 100/10 µg, Aztreonam (ATM) 30 µg and Cefepime (CPM) 10 µg. To *S. aureus* were used Ciprofloxacin (CIP) 5 µg, Cefoxitin (CFO) 30 µg, Gentamicin (GEN) 10 µg, Penicillin (PEN) 10 µg, Clindamycin (CLI) 2 µg, Erythromycin (ERI) 15 µg, Sulfamethoxazole and Trimetoprim associated (SUT) 30 µg, Linezolid (LNZ) 30 µg, Rifampicin (RIF) 5 µg e Tetracycline (TET) 30 µg, as recommended by CLSI[19].

For *S. aureus*, clindamycin resistance induction test, called D test, was performed to detected resistance to macrolides, lincosamines and streptogramins. Was also evaluated the oxacillin resistance *mec*A gene mediated. To evaluate the activity of β-lactamase resistance mediated by *bla*Z gene, was considered the penicillin halo measurement. *Pseudomonas aeruginosa* tests were performed to evaluate the β-lactamases enzymes activity type AmpC, Extended Spectrum β-Lactamase (ESβL), metallo-β-lactamase and carbapenemase, using the disc difusion method, according to ANVISA methodology[15]

Resistance genes amplification

The samples with positive resistance, phenotypic tests were used to plasmid and chromosomal DNA extraction, according Pharmacia® Flexiprep extraction kit manual.

For *S. aureus*, specific primers *bla*Z and *mec*A genes were designed for one of the authors. Was also used primer for *fem*A gene to confirm the *S. aureus* phenotypic identification, according to store sequences in GenBank[20,21].

For *P. aeruginosa*, specific primers for *bla*VIM, *bla*KPC, *bla*SHV, *bla*OXA, *bla*CMY, *bla*IMP, *bla*NDM e blaTEM genes were designed by one of the authors, based on literature [22, 23, 24, 25, 26]. The qPCR technique conditions were performed according to the manufacturer's instructions.

Statistical analysis

For percentage analysis and graphs, Microsoft Excel (Microsoft Corp., Redmond, WA, USA) was used. To associate the data obtained in the antibiogram test for phenotypic resistance and susceptibility, and the presence or absence of the resistance genes observed along with the seasons of the year, the MATLAB script (version 8.1, Natwick, USA) was applied using the test Chi-square.

Results

Among the 11 participants, a total of 88 tracheal secretion samples were obtained and 193 bacterial colonies from 41 different species were isolated, 15 classifieds as gram positive and 26 classified as gram negative. For several patients, there was concomitant isolation of more than one type of bacteria.

Among the gram positive bacteria, we highlight *Corynebacterium* spp., isolated in most patients in all seasons of the year. Coagulase negative *Staphylococcus* (SCN) and *S. aureus* were also isolated in all seasons. From the CNS isolates the following species were identified: *S. epidermidis, S. haemolyticus, S. hominis and S. lugdunensis. Planococcus spp., S. schileiferi and Stomatococcus* spp. were the least prevalent, being found in one patient in spring, autumn and summer respectively (Table 1).

Table 1 – Presence of bacteria in tracheal secretions of tracheostomized children, according to seasons and number of patients.

Gram Positive Microrganisms	Patients Number				Bacteria Total
	Autumn	Winter	Spring	Summer	
Corynebacterium spp.	8	10	8	8	34
SCN	9	3	6	10	28
Staphylococcus aureus	4	3	2	3	12
Bacillus spp.	1	1	0	1	3
Microbacterium spp.	0	0	3	0	3
Staphylococcus hyicus	0	1	2	1	3
Staphylococcus intermedius	0	3	2	2	3
Micrococus spp.	1	0	1	0	2
Planococcus spp.	0	0	1	0	1
Staphylococcus schileiferi	1	0	0	0	1
Stomatococcus spp.	0	0	0	1	1
Total	**24**	**21**	**25**	**26**	**91**

Gram Negative Microrganisms	Patients Number				Bacterias total
	Autumn	Winter	Spring	Summer	
Pseudomonas aeruginosa	9	8	6	7	30
Moraxella spp.	4	1	5	0	10
Citrobacter spp.	2	2	1	1	6
Enterobacter aerogenes	2	1	2	1	6
Klebsiella pneumoniae	1	1	1	1	4
Morganella morganii	0	0	3	1	4
Yersinia aleksiciae	0	1	1	2	4
Enterobacter cloacae	1	2	0	0	3
Escherichia coli	1	1	0	1	3
Hafnia alvei	0	0	2	1	3
Klebsiella oxytoca	1	0	2	0	3
Proteus vulgaris	0	0	1	2	3
Providencia spp.	0	1	2	0	3
Serratia entomophila	0	0	2	1	3
Serratia liquefaciens	1	0	1	1	3
Serratia rubidae	1	0	0	1	2
Sphingomonas spp.	1	1	0	0	2
Stenotrophomonas maltophilia	1	1	0	0	2
Acinetobacter lwoffii	1	0	0	0	1
Enterobacter agglomerans	0	0	1	0	1
Klebsiella ascorbata	0	0	0	1	1
Plesiomonas spp.	1	0	0	0	1
	0	0	0		

Serratia grimesii				1	1
Serratia marcescens	1	0	0	0	1
Serratia odorífera	1	0	0	0	1
Tatumella cítrea	0	0	0	1	1
Total	**29**	**20**	**30**	**23**	**102**

Subtitle: **CNS:** *Coagulase Negative Staphylococcus*.

Analyzing the number and diversity of bacterial species with respect to seasonality, it can be observed that in summer 25 different species of bacteria were isolated, followed by autumn and spring with 24 species and 18 species in winter. As for the number of isolated bacteria, autumn and spring were the seasons with the highest number of isolates, followed by winter and summer (Figure 1).

Observing the diversity individually, it is noteworthy that in three patients[27, 28] the isolated microbiota varied in all seasons in an equalized manner, which shows that the seasons may not have interference in the microbiota diversity (Table 2).

Table 2 – Diversity of bacterial species isolated in tracheal secretions according to each tracheostomized patient.

Patients	Species Diversity			
	Autumn	Winter	Spring	Summer
Paciente 1	4	6	7	4
Paciente 2	2	4	3	6
Paciente 3	3	3	6	A
Paciente 4	6	2	8	A
Paciente 5	3	3	2	4
Paciente 6	7	5	9	6
Paciente 7	5	4	2	5
Paciente 8	6	4	3	6
Paciente 9	8	5	5	6
Paciente 10	5	3	11	4
Paciente 11	8	2	A	8

Subtitle: **A:** Patient absent from collection.

For the biofilm production tests, we chose the species of clinical importance that were most prevalent in the patients and were present in all seasons of the year. Thus, biofilm production was evaluated in 12 isolates of *S. aureus*.

Despite *Corynebacterium* spp. was the most prevalent specie, the phenotypic test was not performed to verify biofilm production, as this bacterium is not commonly associated with respiratory infections. Regarding the CNS, although they were also prevalent, when evaluated separately, they were not isolated in all seasons of the year.

To the experiments using Congo Red Agar, only isolates of *S. aureus* were tested, since the test does not show effectiveness for *P. aeruginosa* samples, including the positive control strain, known as biofilm-forming. All isolates of *S. aureus* (12) were positive in Congo red agar, and therefore all biofilm-forming.

As for biofilm adhesion intensity, *P. aeruginosa* isolated were 50% weakly, 20% moderately and 30% strongly adherent. *Staphylococcus aureus* isolated were 8% weakly, 42% moderately and 50% strongly adherent (Figure 2).

Tests to observe the antimicrobial resistance were performed in 27 *S. aureus* isolates. In the D test, 51.8% (14/27) had a positive result. In the antimicrobial susceptibility test of *S. aureus*, it can be highlighted that among the 27 isolates tested, 100% were resistant to penicillin, 70.4% (19/27) to cefoxitin/oxacillin and 66.7% (18/27) to erythromycin (Figure 3).

Regarding the resistant genes amplification in the *S. aureus* tested samples, 42.8% (3/7) isolates containing *bla*Z in plasmid and chromosomal DNA was identified. The *mec*A gene was amplified in 28.6% (2/7) isolates. These genes were identified in 85.7% (6/7) different isolates. The *fem*A gene was amplified in 100% of the samples (Table 3).

Table 3 – Amplification of *bla*Z, *mec*A and *fem*A genes in *Staphylococcus aureus* samples isolated from tracheostomized children tracheal secretion.

Isolates	Gene Localization	Resistance Genes		
		*bla*Z	*mec*A	*fem*A
1	P	1	0	0
	C	2	2	1
2	P	1	0	0
	C	1	2	1
3	P	2	0	0
	C	2	1	1
4	P	2	0	0
	C	2	2	1
5	P	2	0	0
	C	2	1	1
6	P	1	0	0
	C	1	2	1
7	P	2	0	0
	C	1	2	1

Subtitle: **P:** Plasmidial; **C:** Chromosomal; **0:** no avaliated; **1:** Gene presence; **2:** Gene absences.

For *S. aureus* isolates the phenotypic association occurred between the antibiotic tested cefoxitin with erythromycin and penicillin, all with *p-value* <0.01. There was no statistically phenotypic association with the genotypic results and between the analyzed genes, as well as no variation and statistical association between the seasons and the data obtained.

In the data obtained for *P. aeruginosa*, we can highlight resistance of 23.9% (17/71) for aztreonam and 12.7% (9/71) for imipenem (Figure 4). In the phenotypic test to evaluate the β-lactamase activity, all were negative to ESβL, metallo-β-lactamase and carbapenemase.

Based on the resistance found in *P. aeruginosa* antibiogram test, the literature search showed 12 genes responsible for β-lactams resistance (dates not shown).

In *P. aeruginosa*, of the 12 genes researched, 10 were found in plasmid DNA and 10 in chromosomal DNA. Among the samples tested, the most frequent chromosomal genes were *bla*OXA with 66.7% (8/12), *bla*KPC with 58.3% (7/12), *bla*VIM e *bla*CMY with 41.7% (5/12) of positivity. For plasmid DNA, were highlighted *bla*NDM with 58.3% (7/12), *bla*SHV, *bla*OXA e *bla*TEM with 41.7% (5/12) of positivity (Figure 5).

For the genetic difference identified in each strain of *P. aeruginosa* researched, can highlight the samples “D” with nine different gene types on chromosome and five on plasmid; sample “G” with seven types on chromosome and eight on plasmid; samples “H” with five types on chromosome and seven non plasmid and sample “B” showing seven gene types on chromosome and one on plasmid (Table 4).

Table 4 – Amplification of *Pseudomonas aeruginosa* resistance genes isolated from tracheostomized pediatric patients, according to DNA origin.

Subtitle: **C:** Chromosomal; **P:** Plasmidial; **0:** no avaliated; **1:** Gene absence; **2:** Gene presence.

Source: BUSH; JACOBY (2010).

Isolated	Gene Localization	Resistance Genes												Total of genes
		*bla*SPM	*bla*SIM	*bla*VIM	*bla*KPC	*bla*SHV	*bla*CTX-M	*bla*OXA	*bla*IMP	*bla*NDM	*bla*SME	*bla*CMY	*bla*TEM	
A	C	0	0	1	1	1	1	0	0	0	0	1	1	**0**
B	P	0	0	1	1	2	0	1	0	1	0	1	1	**1**
	C	0	0	2	2	2	2	2	0	2	0	2	1	**7**
C	P	1	1	1	1	0	0	2	1	2	0	0	0	**2**
	C	0	0	2	2	0	0	2	2	1	1	0	0	**4**
D	P	1	0	1	1	2	0	1	2	2	0	2	2	**5**
	C	0	0	2	2	2	2	2	2	2	0	2	2	**9**
E	P	1	1	1	1	0	0	1	1	1	0	0	0	**0**
	C	0	0	1	2	0	0	2	1	1	2	0	0	**3**
F	P	0	0	1	1	1	0	1	0	2	0	1	1	**1**
	C	0	0	1	2	1	1	1	0	2	0	2	2	**4**
G	P	1	0	2	2	2	0	2	2	2	0	2	2	**8**
	C	0	0	2	2	2	2	2	1	1	0	2	2	**7**
H	P	2	1	2	1	2	0	2	1	2	0	2	2	**6**
	C	0	0	2	2	1	2	2	2	1	1	1	1	**5**
I	P	0	0	1	2	2	0	1	0	2	0	1	1	**3**
	C	0	0	1	1	1	1	1	0	1	0	2	1	**1**
J	P	1	0	2	1	1	0	2	2	2	0	1	2	**5**
	C	0	0	1	1	2	1	2	1	2	0	1	2	**4**
K	P	1	1	1	2	0	0	1	2	1	0	0	0	**2**
L	P	0	0	1	1	1	0	1	0	1	0	1	2	**1**
	C	0	0	1	1	1	1	2	0	1	0	1	1	**1**

For *P. aeurginosa* isolates there was no statistical association between the tested antibiotics. The association between the seasons and the antibiotics ceftazidime and imipenem was obtained by the genes *bla*SHV, *bla*CMY and *bla*NDM, with *p-value* <0.05. The gene associations analyzed occurred in *bla*VIM with *bla*SHV, *bla*CTXM, *bla*OXA and *bla*NDM (*p-value* <0.05); *bla*KPC with *bla*CMY; *bla*SHV with *bla*CTX-M, *bla*CMY and *bla*NDM (*p-value* <0.05); *bla*CTX-M with *bla*CMY and *bla*TEM (*p-value* <0.04); and *bla*CMY with *bla*NDM (p-value <0.05).

The statistical association between antibiotics and genes ocurred on cefepime with *bla*IMP; ceftazidime with *bla*VIM, *bla*SHV and *bla*IMP; imipenem with *bla*SHV, *bla*CMY and *bla*TEM (*p-value* <0.05). The results obtained from the phenotypic antibiogram for the aztreonam, gentamicin and Piperacillin / Tazobactam antimicrobials did not show statistically significant association with any of the genes studied in this study.

Discussion

The surgical opening in tracheostomized patients for the device installation causes a breakdown of the skin barrier and causes these microorganisms to become pathogenic[1, 5].

In the present study, of the 27 *S. aureus* isolated, were found resistance that are similar to the study developed by Cavalcanti et al.[29], that found 83.6% (53/123) of resistance to penicillin, 26.2% (16/123) to cefoxitin, 16.4% (10/123) to erythromycin, 11.5% (7/123) to tetracycline, 9.8% (6/123) to clindamycin, and 100% of susceptibility to gentamicin.

To confirm the *S. aureus* specie identified, *fem*A gene was used and was found in 100% of the samples. The phenotypic resistance to penicillin may be confirmed with the *bla*Z gene amplification, even on chromosomal DNA, that plasmidial DNA. The resistance acts in other types of penicillins as amoxicillin, ampicillin and piperacillin. However, stable penicillins such as oxacillin and methicillin, cephalosporins, β-lactamase inhibitors and carbapenems are not included[30, 31].

The methicillin resistance mediated by *mec*A gene can be phenotypically identified by cefoxitin, as identified in this study. This gene is responsible for the synthesis of a modified penicillin binding protein (PBP2a), which interferes with the formation of the bacterial cell wall, preventing its complete structuring[32].

In SCC*mec* it's also possible to find resistance genes for macrolides, quinolones and lincosamines, which have antimicrobial resistance to CIP, ERI, CLI, GEN, among others, increasing the resistance to the group of macrolides, lincosamines and streptogramins, one of the most used in the treatment of staphylococcal infections. However, resistance to these antimicrobials has already been pointed out due to the constant vancomycin use and the easy acquisition of plasmids containing encoded genes to citoplasmatic membrane proteins that acts as efflux pump related to tetracycline[12, 33, 34].

Inducible resistance to clindamycin was found in D test in 51.8% (14/27) of isolates, which limits its effectiveness as a treatment option in MRSA infections. Therefore, the application of D test in the laboratorial routine is useful to investigated possible clindamycin resistance that helps the clinician in the effective use of clindamycin when this was a therapeutic option[35].

For *P. aeruginosa*, in the present study, phenotypic resistance were identified to the antibiotics ATM, IPM, CAZ, PPT, CPM, CIP and GEN, similar to Pires et al.[36] study, that identified resistance to 47.1% (46/87) CIP, 74.4% (21/82) ATM, 45% (74/170) CPM, 46.6% (69/148) GEN and 18.2% (14/77) IPM. The results suggested that *P. aeruginosa* resistance found is due the β-lactamases enzymes production and due the modification of the cell wall outer membrane permeability through the loss or reduction of porins, or by overexpression of efflux pumps present in the plasmatic membrane[37].

In the *P. aeruginosa* samples, the most prevalent resistance genes encoding β-lactamase enzymes were *bla*OXA, *bla*KPC, *bla*VIM, *bla*CMY, *bla*TEM e *bla*SHV. The first two encode carbapenemases enzymes, *bla*VIM encodes metallo-β-lactamases, *bla*CMY ampicillins and the last two ESβL enzymes[37, 38, 39]. The "D" and "H" samples stood out due to the great genetic variation and according to previous bibliographic research, we can observe the variety of antimicrobials that are inactivated when there is the expression of the mentioned enzymes (Table 5).

Table 5 – Correlation of resistance genes for β-lactamases production found in two strains of *Pseudomonas aeruginosa* and the enzyme target antibiotics.

ple	*bla*TEM	*bla*CMY	*bla*NDM	*bla*IMP	*bla*OXA	*bla*CTX-M	*bla*SHV	*bla*KPC	*bla*VIM	
	PEN, PPT, CRO, CPM, ATM, CAZ, AMC, CFO, CFL	PEN, PPT, CRO, ATM, CAZ, AMC, CFO, CFL	ATM, CAZ, AMC, CFO, IPM	CPM, CAZ, IPM	CPM, ATM, CAZ, IPM	PEN, PPT, CRO, CPM, ATM, CAZ, AMC, CFO, CFL	PEN, PPT, CRO, ATM, CAZ, AMC, CFL	PPT, CRO, CPM, ATM, CAZ, CFL, IPM	PPT, CPM, ATM, CAZ, IPM	
ple	*bla*TEM	*bla*CMY	*bla*NDM	*bla*IMP	*bla*OXA	*bla*CTX-M	*bla*SHV	*bla*KPC	*bla*VIM	*bla*SPM
	PEN, PPT, CRO, CPM, ATM, CAZ, AMC, CFO, CFL	PEN, PPT, ATM, CAZ, AMC, CFO, CFL	ATM, CAZ, AMC, CFO, IPM	CPM, CAZ, IPM	CPM, ATM, CAZ, IPM	PEN, PPT, CPM, ATM, CAZ, AMC, CFO, CFL	PEN, PPT, ATM, CAZ, AMC, CFL	PPT, CRO, CPM, ATM, CAZ, CFL, IPM	PPT, CPM, ATM, CAZ, IPM	CAZ, IPM

Subtitle: **PEN:** Penicillin; **PPT:** Piperacillin + Tazobactam; **CRO:** Ceftriaxone; **CPM:** Cefepime; **ATM:** Aztreonam; **CAZ:** Ceftazidime; **AMC:** Amoxicillin + Clavulanic Acid; **CFO:** Cefoxitin; **CFL:** Cephalothin; **IPM:** Imipenem.

Analyzing the amplitude of each enzyme resistance, the samples can be considered multiresistant[40], because the genes simultaneous expression is capable of inactivate the action of AMC and PPT antimicrobials belonging to the class of β-lactams, from first generation of cephalosporins to fourth generation[32].

The antibiogram tests results didn't have phenotypical resistance to the tested antibiotics. This divergence occurs, because only the presence of the gene responsible for antimicrobial resistance isn't automatically linked to its expression[41].

The antimicrobials β-lactams are the most used in bacterial respiratory tract infections, because they are highly effective and low toxicity[42]. Bellés et al.[43] identified that of the 160 patients studied (adults and children), 80 did antibiotic therapy with β-lactams, which in 16 of these was identified metallo-β-lactamase enzyme activity. In addition, national epidemiological studies evaluated 3728 gram positive and gram negative isolates, and *P. aeruginosa* was responsible for 496 (13.3%) cases, being the third pathogen most frequent that presented 30.2% of IPM resistance[44].

Carbapenems are the most important antibiotics in the treatment of multiresistant *P. aeruginosa* infections. The resistance granted to this class makes treatment difficult[11, 45].

Regarding seasonality, there was no significant variation in the diversity of the microbiota throughout the year. This finding was similar to that described by Perez-Losada et al.[46], Who also did not observe a

difference in the tracheal secretion microbiota during the seasons.

It was expected to find changes in the microbiota mainly in winter, but the absence of this variation can be explained by the location of the tube, found in the trachea whose colonization differs when compared to the respiratory tract infections, where there is in fact a microbiological diversity that can be influenced by seasonality, due to greater contact with the external environment, as well as the presence of anatomical structures that contribute to microbiological control[47, 48].

The data from this study confirm that there is a relationship of infections occurring in lower respiratory tract of tracheostomized patients, with resistant bacteria. The cannula microbiota is influenced by the care of device hygiene and its permanence, when used for a long period, can lead to tracheal mucosa inflammation, increasing the risks of infections[1, 47, 49].

Taechowisan et al.[50] verified the relationship between the phenotypic and genotypic resistance and found that those based on PCR did not completely correlate with the phenotypic resistance, which was also found in this study of bacterial isolates from tracheostomized children. Mohaman and Menon[51] also find coexistence of *bla* genes for metallo-β-lactamases in *P. aeruginosa*.

Researchers suggest the importance of changing the device regularly, since the patients in the present study performed this change once a month, and even so, a diversity of microorganisms was found, as well as biofilm-forming bacteria. Thus, if we consider patients who do not change the cannula frequently, they will probably present an intense and prolonged colonization, making the device a reservoir of bacteria, with the formation of persistent biofilm[52].

In Brazil, tracheostomy tubes are not available free of charge by the Public Health System, making patients pay with the purchase of the device and often do not have the financial resources to do so, which makes monthly changes difficult[52]. In addition, the chances of biofilm adherence increase, resulting in the formation of granulation tissue, recurrent infections and failure in laryngotracheal reconstruction[53].

Prolonged intubation and upper airway obstruction are frequent indications for tracheostomy, as well as acute respiratory failure and neurological disorders or injuries, which, according to some authors, are the least usual indications[54, 55, 56]. Regarding age, several studies show that tracheostomy is performed more frequently in pediatric patients, with higher rates being observed in children under one year old, newborns and premature, thus increasing the survival of this public[56, 57, 58, 59, 60, 61].

Due to the increased survival, tracheostomy has become a constant practice for those children who need mechanical ventilation, but the initial management is with endotracheal intubation. However, this intubation for long periods brings several problems to the patient, such as mucosal ulcers and ischemia of the larynx or trachea, which justifies the commonly indicated tracheostomy[58].

The knowledge of the predominant microbiota in lower respiratory tract infections as a result of device use in tracheostomized patients, as well as the resistance profile of the most antimicrobial used, will help

health professionals regarding the most appropriate therapy to be given children with traqueostomy, especially in the postoperative period, increasing the chances of airway restoration and decannulation, since local hygiene is not always performed as recommended[27].

Conclusion

The *S. aureus* phenotypic tests identified resistance to β-lactams groups, macrolides, lincosamines, aminoglicosides and tetracyclines. In *P. aeruginosa*, the phenotypic tests identified resistence to all antibiotics tested.

Resistance genes as *bla*Z e *mec*A in S. aureus and genes *bla*TEM, *bla*NDM, *bla*OXA, *bla*CMY, *bla*IMP, *bla*CTX-M, *bla*SHV, *bla*KPC, *bla*VIM and *bla*SPM in *P. aeruginosa*. The amplified genes are correlated with the antibiogram data.

Declarations

Ethics approval and consent to participate

The research project was submitted and approved by the Ethics Committee of the Clinical Hospital of the Federal University of Goiás (CEP/HC/UFG), by number 32091014.6.1001.5078.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors have made substantial contributions to the conception and design of the work, contributions with acquisition analysis, interpretation of data and have revised it. Besides, all authors have approved the submitted version (and any substantially modified version that involves the author's contribution to the study), and have agreed both to be personally accountable for the author's own contributions and to

ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Footnotes

Not applicable.

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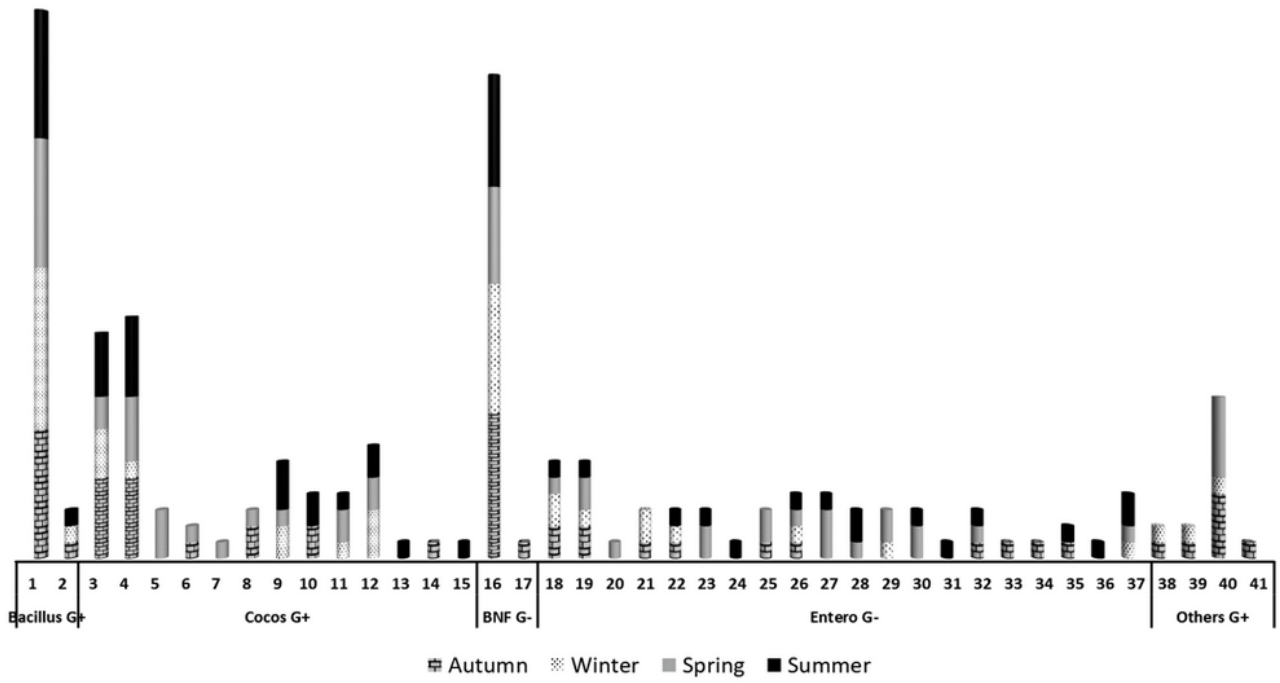
Figures



1	*Corynebacterium* spp.	15	*Stomatococcus* spp.	29	*Providencia* spp.
2	*Bacillus* spp.	16	*Pseudomonas aeruginosa*	30	*Serratia entomophila*
3	*Staphylococcus aureus*	17	*Acinetobacter lwoffii*	31	*Serratia grimesii*
4	*Staphylococcus coagulase* negativa	18	*Citrobacter* spp.	32	*Serratia liquefaciens*
5	*Microbacterium* spp.	19	*Enterobacter aerogenes*	33	*Serratia marcescens*
6	*Micrococus* spp.	20	*Enterobacter agglomerans*	34	*Serratia odorifera*
7	*Planococcus* spp.	21	*Enterobacter cloacae*	35	*Serratia rubidae*
8	*Staphylococcus epidermidis*	22	*Escherichia coli*	36	*Tatumella citrea*
9	*Staphylococcus haemolyticus*	23	*Hafnia alvei*	37	*Yersinia aleksiciae*
10	*Staphylococcus hominis*	24	*Klebsiella ascorbata*	38	*Sphingomonas* spp.
11	*Staphylococcus hyicus*	25	*Klebsiella oxytoca*	39	*Stenotrophomonas maltophilia*
12	*Staphylococcus intermedius*	26	*Klebsiella pneumoniae*	40	*Moraxella* spp.
13	*Staphylococcus lugdunensis*	27	*Morganella morganii*	41	*Plesiomonas* spp.
14	*Staphylococcus schileiferi*	28	*Proteus vulgaris*		

Figure 1

Diversity of bacterial species isolated from tracheal secretions of tracheostomized children according to seasons. Subtitle: G+ Gram positives; BNF G- No fermentative gram negative Bacillus; Others G- Others gram negative bacteria.

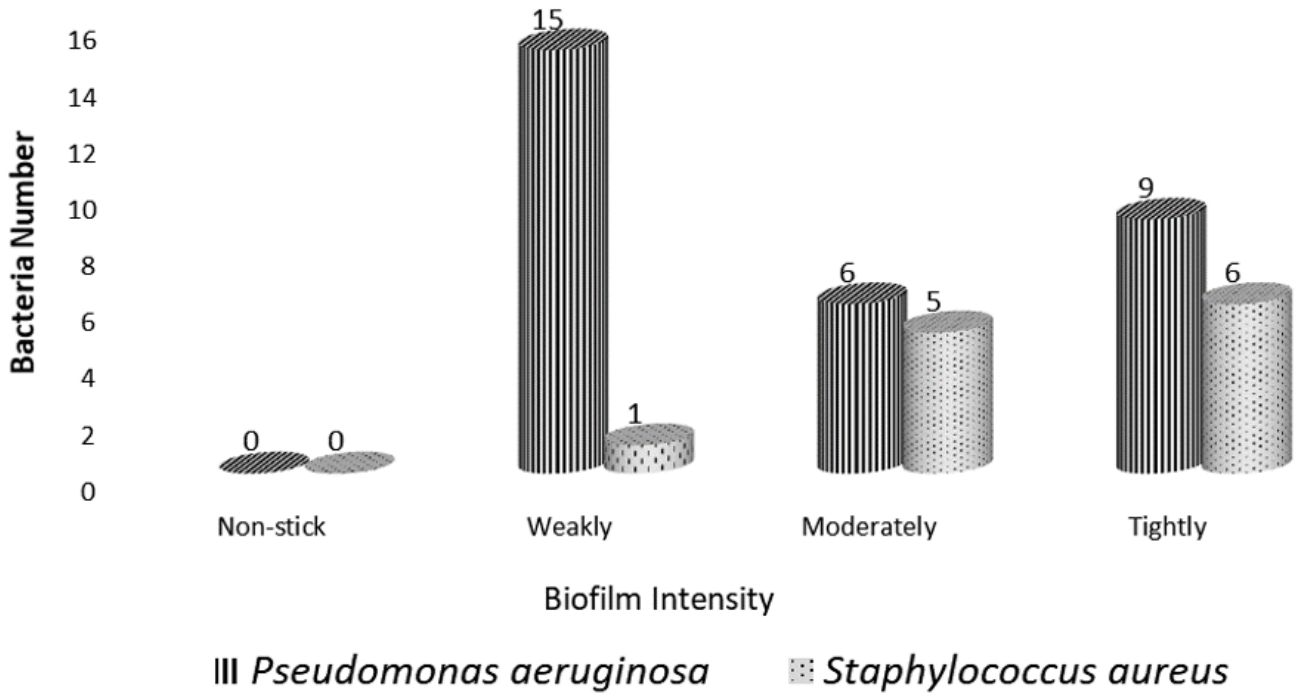


Figure 2

Adhesion intensity of the biofilm of Pseudomonas aeruginosa and Stahylococcus aureus from the tracheal secretions of traqueostomized children.

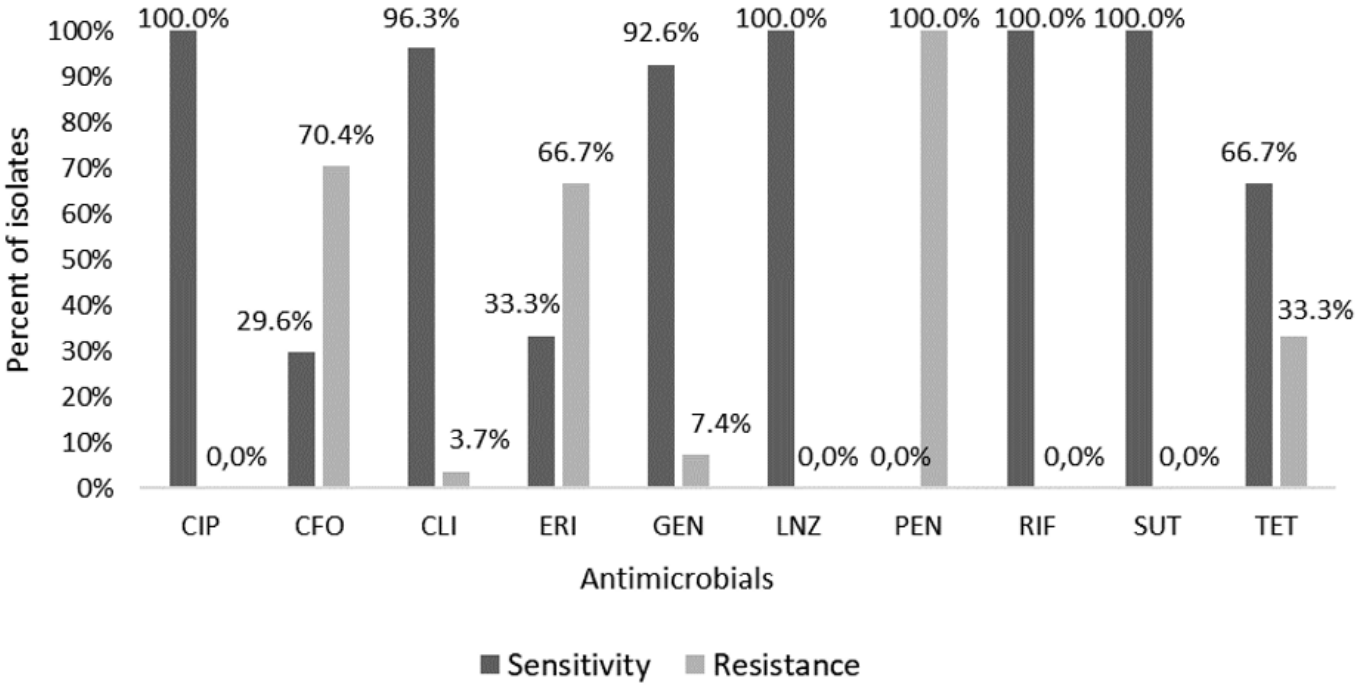


Figure 3

Phenotypic resistance profile of Staphylococcus aureus isolated from tracheostomized pediatric patients' tracheal secretion. Subtitle: CIP: Ciprofloxacin; CFO: Cefoxitin; CLI: Clindamycin; ERI: Erythromycin; GEN: Gentamicin; LNZ: Linezolide; PEN: Penicillin; RIF: Rifampicin; SUT: Sulfamethoxazole + Trimetoprim; TET: Tetracycline.

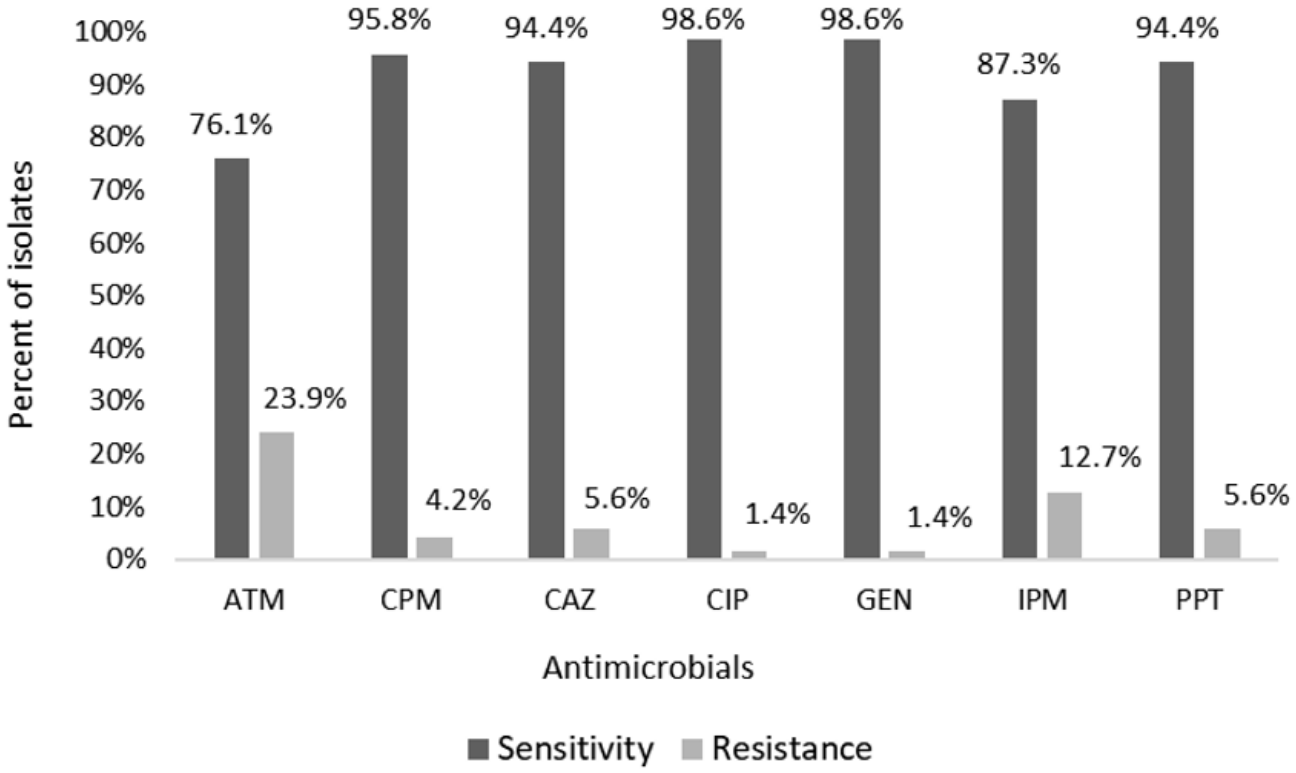


Figure 4

Phenotypic resistance profile of Pseudomonas aeruginosa isolated from tracheostomized pediatric patients' tracheal secretion. Subtitle: ATM: Aztreonam; CPM: Cefepime; CAZ: Ceftazidime; CIP: Ciprofloxacin; GEN: Gentamicin; IPM: Imipenem; PPT: Piperacillin + Tazobactam.

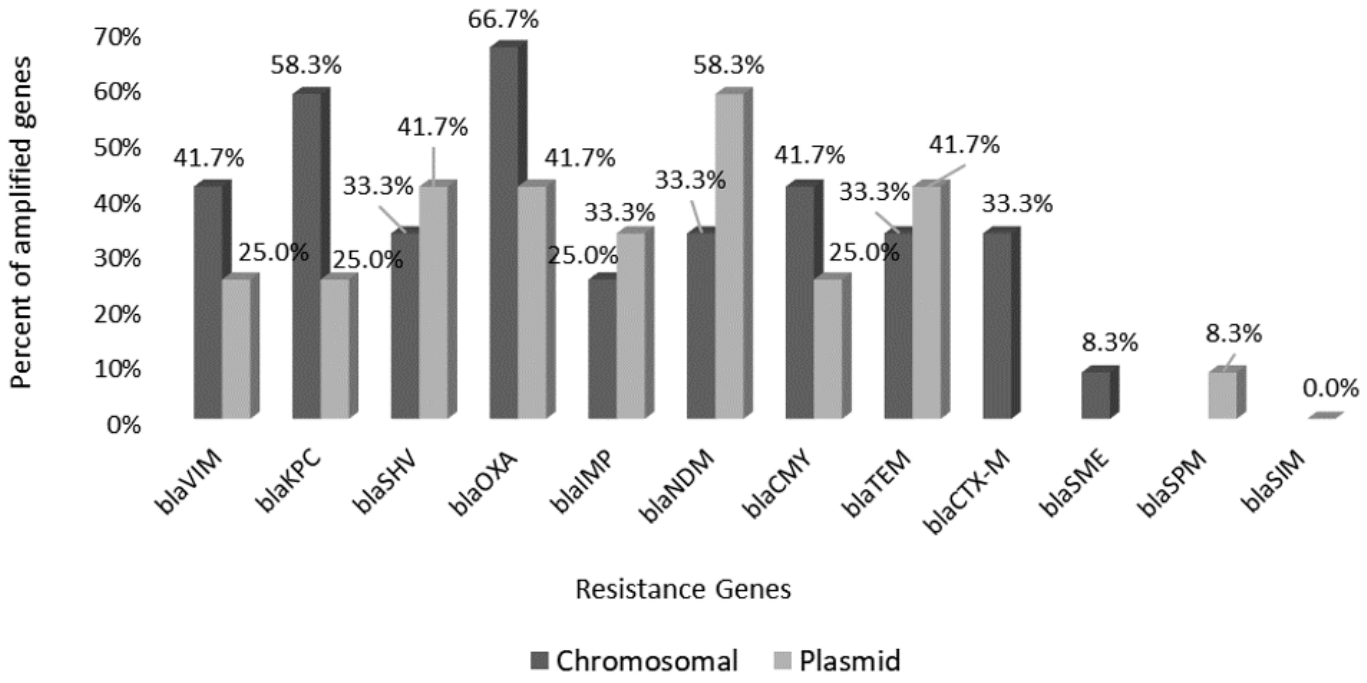


Figure 5

Resistance genes amplification in Pseudomonas aeruginosa samples isolated from tracheostomized pediatric patients.