

In Vitro Antibiotic Sensitivity and Resistance of 100 Clinical Bacterial Isolates Purified from Microbial Biofilms Associated with Silicone Gastrostomy Tubes Removed from Pediatric Patients

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ABSTRACT

In vitro analysis of 7 antibiotics against 100 clinical bacterial isolates enriched from pediatric gastrostomy tubes was performed in this study. Various gram-positive and negative organisms were purified from percutaneous pediatric feeding tubes and assayed for antibiotic susceptibility and resistance. A total of 10 gram-negative isolates, predominantly *Escherichia coli*, and 90 gram-positive organisms, mostly belonging to the *Staphylococcus* and *Enterococcus* genera were examined for antimicrobial resistance and sensitivity. Seven antibiotics, which included ampicillin, cefazolin, ceftriaxone, ciprofloxacin, gentamicin, oxacillin, and vancomycin, were tested based on their possible use in pediatric patients requiring feeding tubes for nutritional support. Minimum inhibitory concentrations were determined for all isolates and their relative resistance profiles were generated. The

Staphylococcus genus possessed the highest diversity for antibiotic resistance while organisms comprising the *Enterococcus* genus exhibited marginal levels of resistance to the antibiotics tested in this study. Approximately 43% of the isolates tested displayed multiple drug resistance, with the predominant species belonging to the *Staphylococcus* genus. This investigation reports the effectiveness of 7 commonly used antibiotics on various microbial species that are capable of initiating and maintaining bacterial biofilms on surgically implanted feeding tubes.

INTRODUCTION

The human body provides an ideal environment for bacterial colonization and growth. However, in most circumstances, host innate and acquired immunological responses control this degree of bacterial proliferation and attachment.¹ Bacteria have adapted complex mechanisms for persisting in unfavorable environments commonly encountered in host tissues and on vari-

Table 1. MIC Values for 10 Isolates from the Enterobacteriaceae Family

Isolate*	MIC ($\mu\text{g/mL}$)						
	AMP	CFZ	CRO	CIP	GEN	OXA	VAN
Ec3	5	25	10	1	116	302	>1000
Ec9A	10	25	>1000	1	96	202	>1000
Ec9B	5	40	>1000	1	116	138	>1000
Ec9C	10	21	10	1	116	176	>1000
Ec9D	10	38	>1000	1	6	301	>1000
Ec9E	>1000	61	496	82	>1000	505	>1000
Ec9F	10	40	>1000	1	116	201	>1000
Kp7	>1000	53	549	2	106	404	>1000
Pm18	30	110	30	1	140	>1000	>1000
Ye10	4	15	5	1	2	5	16

*Isolate code based on organism (Ec, *E. coli*; Kp, *K. pneumoniae* subsp. *pneumoniae*; Pm, *P. mirabilis*; Ye, *Y. enterocolitica*), patient number, and designated with a letter if multiple isolates were from a single patient.
AMP indicates ampicillin; CFZ, cefazolin; CRO, ceftriaxone; CIP, ciprofloxacin; GEN, gentamicin; OXA, oxacillin; and VAN, vancomycin.

ous inert surfaces.² Insertion of prosthetic devices, especially in immunocompromised patients or the young and elderly, often leads to the formation of microbial biofilms.³ When bacterial cells form a biofilm, complement-mediated opsonizing factors and phagocytic cells are not always capable of eradicating the colonizing organisms.⁴ Furthermore, antimicrobial agents tend to be less effective against a community of various microorganisms embedded in an established biofilm when compared to individual strains grown in conventional suspension cultures.⁵ However, as acknowledged by Monzón et al, the need to decipher the specific antibiotic resistance or susceptibility profiles of the individual bacteria comprising biofilms is essential for reducing associated infections.⁶

Biofilm linked infections have led to research focusing on the mechanism behind the cells' reduced susceptibility to antimicrobial agents.^{2,3,7-10} The most scientifically accepted hypothesis for this phenomenon suggests that adherent microorganisms (sessile bacteria) are protected from fluctuating environmental conditions by growing in organized

communities encompassed in carbohydrate and exopolysaccharide matrices.² These organisms are subjected to nutrient limitations and thus have slower generation times in contrast to planktonic (free-floating) cells, which have an increased generation time and have more access to essential growth factors.^{2,8} Sessile bacteria, located within the inner domain of the biofilm, may not be exposed to effective amounts of antibiotics while planktonic (organisms sloughing from the biofilm) organisms are easily accessed and killed by antibacterial agents at lower concentrations. The protective environment provided by microbial biofilms establishes various gradients for nutrient and antibiotic penetration. The failure of adequate antibiotic levels to reach sessile organisms has been shown to lead to recurring infections, especially in patients with surgically implanted devices.^{2,8,9,11}

Limiting nutrients cause sessile bacteria to switch to a slow-growing or even a starvation-like state. These metabolic phases have been shown to enhance the cells' resistance to antimicrobial and chemical agents.¹² It has been demonstrated that thin (2-day old) biofilms

Table 2. MIC Values for 33 Isolates from the Genus *Enterococcus*

Isolate*	MIC ($\mu\text{g/mL}$)						
	AMP	CFZ	CRO	CIP	GEN	OXA	VAN
Ed10A	>1000	25	742	1	116	4	1
Ed10B	2	201	546	1	116	5	1
Ed15	2	16	445	1	96	4	1
Ef2A	2	381	10	1	191	5	1
Ef2B	2	25	10	1	186	5	1
Ef2C	4	325	>1000	1	186	30	9
Ef5A	2	25	10	21	186	5	2
Ef5B	2	25	10	96	>1000	4	1
Ef5C	2	22	10	91	>1000	4	2
Ef5D	2	22	10	96	>1000	5	1
Ef5E	2	21	10	81	>1000	5	1
Ef5F	2	26	10	1	>1000	5	2
Ef5G	2	23	>1000	2	186	5	1
Ef5H	2	41	10	89	>1000	5	2
Ef5I	2	25	10	96	191	4	1
Ef7A	1	25	>1000	1	111	5	1
Ef7B	2	16	10	1	111	12	2
Ef7C	2	25	>1000	1	301	11	2
Ef7D	5	42	>1000	1	96	5	5
Ef14	1	23	686	1	116	5	1
Ef15A	1	10	10	1	116	5	2
Ef15B	2	15	>1000	1	151	5	2
Ef15C	1	23	>1000	1	116	5	2
Efm15A	5	24	551	1	116	90	1
Efm15B	4	200	>1000	1	116	80	1
Efm15C	3	297	934	1	176	501	1
Efm15D	2	25	>1000	1	106	90	1
Efm15E	2	208	10	1	452	501	1
Efm15F	5	211	945	1	116	611	1
Efm15G	1	23	945	1	116	501	1
Efm15H	1	14	500	1	116	421	1
Eh15A	2	194	10	1	116	5	1
Eh15B	2	291	544	1	116	4	1

*Isolate code based on organism (Ed, *E. durans*; Ef, *E. faecalis*; Efm, *E. faecium*, Eh, *E. hirae*), patient number, and designated with a letter if multiple isolates were from a single patient.
AMP indicates ampicillin; CFZ, cefazolin; CRO, ceftriaxone; CIP, ciprofloxacin; GEN, gentamicin; OXA, oxacillin; and VAN, vancomycin.

with uniform growth rates are affected by antibiotics while thicker (7-day old) biofilms contain regions of severely retarded growth that survive antibiotic challenge.¹¹ Natural and acquired microbial resistance to antibiotics has become a global threat in the past few decades, especially in industrialized and

developing countries.¹³ Misdiagnosis, overuse, and lack of education are all contributing factors to this widespread problem.

Reduced antibiotic susceptibility within bacterial biofilms provides organisms with an ecological niche that enhances the risk of infections, especial-

Table 3. MIC Values for 30 Isolates from the Genus *Staphylococcus*

Isolate*	MIC (µg/mL)						
	AMP	CFZ	CRO	CIP	GEN	OXA	VAN
Sa1A	2	15	10	1	6	2	3
Sa1B	2	25	10	1	8	2	2
Sa1C	2	26	10	1	116	2	2
Sa1D	1	24	10	1	4	1	2
Sa1E	601	210	10	1	191	1	2
Sa1F	70	383	10	1	191	3	2
Sa7A	>1000	9	10	1	31	3	1
Sa7B	3	276	10	1	191	1	1
Sa7C	2	27	10	50	>1000	3	1
Sa7D	2	3	7	1	4	1	5
Sa9A	4	28	>1000	1	99	11	1
Sa9B	5	40	>1000	1	106	1	2
Sa9C	4	239	>1000	1	251	5	>1000
Sa9D	2	14	>1000	1	116	1	1
Sa9E	150	15	>1000	1	4	3	2
Sa9F	2	32	10	1	2	1	1
Sa9G	10	38	>1000	1	95	1	1
Sa9H	175	170	804	1	5	1	1
Sa9I	10	38	>1000	1	116	2	1
Sa9J	2	10	10	1	2	2	1
Se10A	2	17	>1000	1	116	5	1
Se10A	2	21	845	1	1	1	3
Si7	2	2	7	1	3	1	2
Ss9A	2	29	10	1	4	1	>1000
Ss9B	2	26	>1000	1	3	1	1
Ss9C	10	40	>1000	1	91	5	1
Ss9D	5	210	>1000	1	116	9	50
Ss9E	2	27	>1000	1	4	1	1
Ss9F	10	40	>1000	1	101	1	2
Ss9G	2	2	10	1	1	1	2

*Isolate code based on organism (Sa, *S. aureus*; Se, *S. epidermidis*; Si, *S. intermedius*, Ss, *S. saprophyticus*), patient number, and designated with a letter if multiple isolates were from a single patient.
AMP indicates ampicillin; CFZ, cefazolin; CRO, ceftriaxone; CIP, ciprofloxacin; GEN, gentamicin; OXA, oxacillin; and VAN, vancomycin.

ly in individuals with surgically implanted medical devices.^{3,14} Antimicrobial resistance in association with biofilms attached to medical devices has the potential to pose a serious threat to the patient. In this study, 100 bacterial biofilm isolates from 16 pediatric patients with surgically implanted percutaneous feeding tubes were tested

against 7 antibiotics. The classes of antibiotics tested belong to the penicillins, cepheims, fluoroquinolones, aminoglycosides, and glycopeptides. Antibiotic sensitivity and resistance profiles were determined and numerous multiple-drug resistant bacteria were identified.

Table 4. MIC Values for 27 Isolates from the Genera *Actinomyces*, *Corynebacterium*, *Bacillus*, *Lactobacillus*, and *Micrococcus*

Isolate*	MIC (µg/mL)						
	AMP	CFZ	CRO	CIP	GEN	OXA	VAN
Miscellaneous gram-positive bacillus							
Ap9	5	2	5	1	50	1	6
Ca17	1	31	10	1	90	6	3
Cp4	6	5	1	1	110	7	3
Genus <i>Bacillus</i>							
Bb9A	71	31	12	1	31	7	3
Bb9B	10	5	4	1	17	1	3
Bl4	122	1	4	1	1	1	5
Bl11A	16	26	30	1	50	6	8
Bl11B	11	2	4	1	90	1	1
Bl16	1	26	25	1	90	1	3
Bm10	5	2	2	1	1	1	6
Bp1A	4	100	5	1	1	1	3
Bp1B	15	2	14	1	1	1	1
Bs5	1	19	5	1	31	16	3
Genus <i>Lactobacillus</i>							
Lp18	1	6	16	10	160	5	>1000
Lp20A	1	2	1	160	30	2	>1000
Lp20C	1	9	1	100	10	3	>1000
Lp20D	1	9	1	100	30	2	>1000
Lp20E	1	9	1	100	10	4	>1000
Lp20F	1	9	1	95	30	3	>1000
Lp20G	1	7	1	100	35	1	>1000
Genus <i>Micrococcus</i>							
Mk5	2	15	10	1	96	1	1
MI9	5	28	>1000	1	96	5	1
Ms6	1	25	10	90	4	1	1
Ms10A	1	10	10	1	96	7	1
Ms10B	>1000	25	346	1	96	299	2
Ms10C	5	32	>1000	2	106	5	2

*Isolate code based on organism (Ap, *A. pyogenes*; Ca, *C. aquaticum*; Cp, *C. pseudodiphtheriticum*; Bb, *B. brevis*; Bl, *B. licheniformis*; Bm, *B. megaterium*; Bp, *B. pumilus*; Bs, *B. subtilis*; Lp, *L. plantarum*; Mk, *M. kristinae*; MI, *M. luteus*; Ms, *M. sedentarius*), patient number, and designated with a letter if multiple isolates were from a single patient.
AMP indicates ampicillin; CFZ, cefazolin; CRO, ceftriaxone; CIP, ciprofloxacin; GEN gentamicin; OXA oxacillin; and VAN, vancomycin.

MATERIALS AND METHODS

Isolation and Identification of Biofilm Microorganisms

From 1998 to 1999, 100 clinical isolates were removed from 16 silicone rubber

low-profile percutaneous endoscopic gastrostomy tubes collected from pediatric patients being treated at The Children's Hospital of Greenville Hospital System in Greenville, SC.

Table 5. Percent Resistance According to MIC Values (NCCLS Guidelines)

AMP Species	No. of isolates	AMP	CFZ	CRO	CIP	GEN	OXA	VAN
<i>Enterococcus faecalis</i>	20	0	*	*	35	30	*	0
<i>Staphylococcus aureus</i>	20	...	45	40	5	65	10	5
<i>Enterococcus faecium</i>	8	0	NA	NA	0	0	NA	0
<i>Escherichia coli</i>	7	14	57	71	14	86	NA	NA
<i>Staphylococcus saprophyticus</i>	7	...	43	71	0	43	100	29
<i>Enterococcus durans</i>	3	33	NA	NA	0	0	NA	0
<i>Enterococcus hirae</i>	2	0	NA	NA	0	0	NA	0
<i>Staphylococcus epidermidis</i>	2	...	0	100	0	50	100	0
<i>Klebsiella pneumoniae</i>	1	100	100	100	0	100	NA	NA
<i>Proteus mirabilis</i>	1	0	100	0	0	100	NA	NA
<i>Staphylococcus intermedius</i>	1	...	0	0	0	0	100	0
<i>Yersinia enterocolitica</i>	1	0	0	0	0	0	NA	NA
Totals	73	7	45	53	12	43	40	5

NA indicates no NCCLS guidelines are available for these organism/antibiotic pairs
 Ellipses indicate additional testing to determine resistance was not performed
 AMP indicates ampicillin; CFZ, cefazolin; CRO, ceftriaxone; CIP, ciprofloxacin; GEN, gentamicin; OXA, oxacillin; and VAN, vancomycin.

Portions of the gastrostomy tubes including the inner and outer regions of the internal stabilizer, shaft, and valve, if present, were scraped with a sterile scalpel to remove viable biofilm mass and cultured as described by Dautle et al.¹⁵ Bacterial isolates were initially classified based on Gram stain and further identified using methods described by Dautle et al.¹⁵

Antibiotic Susceptibility Testing

Seven antibiotics were selected based on their potential use in pediatric patients with gastrostomy devices and include ampicillin, cefazolin, ceftriaxone, ciprofloxacin, gentamicin, oxacillin, and vancomycin. Susceptibility to antimicrobial agents was determined by the disc diffusion method according to the NCCLS guidelines.¹⁶ Discs were purchased from Becton Dickinson (Sparks, Md) and the following disc concentra-

tions were used: ampicillin, 10 µg; cefazolin, 30 µg; ceftriaxone, 30 µg; ciprofloxacin, 5µg; gentamicin, 10 µg; oxacillin, 1 µg; and vancomycin, 30 µg. Zones of inhibition were measured after 18 to 24 hours of incubation at 37°C. Susceptibility breakpoints were determined according to NCCLS guidelines and used to determine ranges for MIC testing.¹⁷

MIC Determination

The antibiotics used for analyzing MICs were as follows: ampicillin (Sigma, St. Louis, Mo), cefazolin (ICN Biomedicals, Aurora, Ohio), ceftriaxone (Sigma), ciprofloxacin (Serologicals Proteins, Kankakee, Ill), gentamicin (Sigma), oxacillin (Sigma), and vancomycin (Sigma). Stock solutions of each drug were prepared in water, filter sterilized (0.2 µm filter), and added to BHI agar (Difco, Detroit, Mich) for all isolates

except *Lactobacillus plantarum*, in which BHI was substituted with MRS agar (Difco). The ranges determined from the disc diffusion assays were used as a starting point for MIC analysis; however, tests were not conducted above an antibiotic concentration of 1,000 µg/mL, which exceeds the therapeutic dose used for human treatment. Antibiotic MIC testing was performed in triplicate as described by Wiggins¹⁸ with the following modifications. Isolates were inoculated into 96-well plates containing 200 µL per well of BHI broth (Difco) for all isolates except *L. plantarum*, where wells contained 200 µL of MRS broth (Difco). The isolates were transferred with a 96-prong replica-plater (Genetix Limited, Dorset, UK) from a source plate to a set of 96-well plates containing the appropriate medium and antibiotic. Plates were incubated for 24 hours at 37°C and visually observed for inhibition or growth in the presence of each antibiotic. An isolate was considered to be resistant to a given concentration of antibiotic if growth occurred in that well. A plate, containing either BHI or MRS agar without antibiotic, was replica-plated in each experiment to provide a positive control.

RESULTS

MIC values and relative susceptibilities for 7 antibiotics were determined for 100 clinical isolates classified within several microbial genera. Organisms belonging to the Enterobacteriaceae family included *Escherichia coli*, *Klebsiella pneumoniae* subsp. *pneumoniae*, *Proteus mirabilis*, and *Yersinia enterocolitica* (Table 1). According to NCCLS guidelines, MIC determinations showed that 20% were resistant to ampicillin, 60% to cefazolin and ceftriaxone, 10% to ciprofloxacin, and 80% to gentamicin. The relative oxacillin MIC ranged from 5 µg/mL to >1000 µg/mL, and that for vancomycin

was 16 µg/mL to >1000 µg/mL (Table 1). Of the Enterobacteriaceae tested against vancomycin, 90% of the organisms were resistant to concentrations >1000 µg/mL.

Species within the *Enterococcus* genus included *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Enterococcus hirae*. Antibiotic resistance and MIC profiles for these isolates are reported in Table 2. Our analysis demonstrated that 3% of the enterococci were resistant to ampicillin, 21% to ciprofloxacin, 18% to gentamicin and 0% to vancomycin based on NCCLS cut off points. Cefazolin MICs ranged from 10 µg/mL to 381 µg/mL, ceftriaxone varied from 10 µg/mL to >1000 µg/mL, and oxacillin varied from 4 µg/mL to 611 µg/mL (Table 2).

In addition to enterococci, MICs and antibiotic susceptibilities for *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus intermedius*, and *Staphylococcus saprophyticus* were determined (Table 3). None of the isolates comprising this experimental group fell below the NCCLS guideline for susceptibility to ampicillin (<0.25 µg/mL). Approximately 40% were resistant to cefazolin, 50% to ceftriaxone, 3% to ciprofloxacin, 57% to gentamicin, and 10% to vancomycin. Oxacillin resistance was categorized into 2 groups consisting of coagulase-positive and coagulase-negative organisms. The 20 coagulase-positive (*S. aureus*) isolates showed 10% resistance to oxacillin while the coagulase-negative organisms (*S. epidermidis*, *S. intermedius*, and *S. saprophyticus*) were 100% resistant. Due to the complex growth requirements of various microbial species, definitive NCCLS cutoff values for antibiotic susceptibility and resistance have not been established. Several isolates in this study fall into this category and have no standardized NCCLS MIC values. This experimental group is comprised of *Actinomyces pyogenes*,

Corynebacterium aquaticum, *Corynebacterium pseudodiphtheriticum*, *Bacillus brevis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus subtilis*, *L. plantarum*, *Micrococcus kristinae*, *Micrococcus luteus*, and *Micrococcus sedentarius*. The preliminary MIC ranges for these organisms are listed in Table 4.

The most frequent antibiotic resistances for all isolates in this survey were observed to cefazolin (45%), ceftriaxone (53%), gentamicin (43%), and oxacillin (40%) (Table 5). Numerous organisms exhibited lower levels of resistance to ampicillin (7%), ciprofloxacin (12%), and vancomycin (5%) (Table 5). Several staphylococci, Enterobacteriaceae, and enterococci isolates exhibited multiple drug resistance with an overall percentage of 43%.

DISCUSSION

The intrinsic physiological and phenotypic fluctuation of microorganisms comprising biofilms plays an essential role in the increased resistance to antimicrobial agents.⁸ Interestingly, 65% of nosocomial infections stem from bacterial species comprising biofilms, costing the global health care system millions of dollars annually.² Surveillance investigations of this type, especially in potentially immunocompromised pediatric patients, are essential for providing pediatricians insight into the relative antibiotic susceptibilities of bacteria associated with percutaneous feeding tubes. Currently, limited investigations have focused on the antibiotic susceptibilities of microorganisms colonizing pediatric feeding tubes. The results of this study will provide pediatricians with a general spectrum of effective antibiotics against gastrostomy associated biofilms and may facilitate the treatment of possible secondary infections associated with biofilms, especially for patients relying on enteral

access tubes.

Within the family Enterobacteriaceae, antibiotic resistance varied and 60% of the isolates tested were resistant to the cepheims (cefazolin and ceftriaxone). It has been reported that this occurrence is a direct result of extensive antibiotic use by the medical community, specifically hospitals.¹⁹ The most effective antibiotic against Enterobacteriaceae was ciprofloxacin and only one isolate (Ec9E) demonstrated resistance (Table 1).

The genus *Enterococcus* was the most susceptible to the antibiotics tested in this work. All isolates were affected by vancomycin and only Ed10A exhibited resistance to ampicillin (Table 2). The highest proportion of resistance among enterococci isolates was to ciprofloxacin and gentamicin, and interestingly, all of these isolates originated from the same patient (Table 2). These isolates were purified from multiple locations on the feeding tube (outer portion of the internal stabilizer, inner and outer portion of the shaft, and valve) and have been determined to be genetically different.¹⁵ Considering the genetic diversity of these organisms, it is conceivable the resistances observed against ciprofloxacin and gentamicin are the result of horizontal gene transfer and do not result from the proliferation of a single organism throughout the biofilm. Several investigations have demonstrated that biofilms provide an ideal environment for the exchange of genetic material.²⁰⁻²³ With regard to those findings, and because each ciprofloxacin and gentamicin resistant enterococcus isolate is genetically distinct, it seems probable that the origin of this resistance may have been acquired via genetic exchange.

The most resistant microbes to the antibiotics analyzed in this investigation belong to the *Staphylococcus* genus. Ampicillin, cefazolin, and ceftriaxone

showed the lowest inhibitory profiles with 0%, 10%, and 7% of the isolates being susceptible, respectively (Table 3). Ten percent of the isolates were determined to be methicillin resistant *S. aureus* (MRSA) by oxacillin susceptibility testing. MRSA strains were also tested for ciprofloxacin susceptibility, which is reported to be effective against MRSA²⁴ and all isolates assayed were indeed ciprofloxacin sensitive.

No NCCLS standards have been determined for the genera *Actinomyces*, *Corynebacterium*, *Bacillus*, *Lactobacillus*, and *Micrococcus* due to non-reproducible results. Despite these limitations, and because some of these species have been implicated with opportunistic infections in immunocompromised pediatric patients, MIC, antibiotic resistance, and susceptibility profiles were determined. Higher concentrations of antibiotics than would be administered for pediatric or adult patients were required to inhibit the growth of many of these isolates (Table 4). The most effective antibiotics against microorganisms within these groups were ciprofloxacin, ampicillin, and vancomycin (Table 4).

Several complications including peritonitis and deep wound infections have been associated with gastrostomy tubes.²⁵ Generally, gastrostomy associated infections are treated with intravenous, oral, and topical antibiotic therapies.²⁵ In the case of extreme infections, patients may require the surgical removal of the enteral access tube.²⁵ Unfortunately, in this study, our laboratory was unable to obtain detailed records and access to patient histories and medical files resulting from patient confidentiality. It should be noted, failure to obtain patient records, specifically previous or current antibiotic therapy, might skew the relative sensitivity and resistance profiles reported in this investigation. Also, the biofilm purified isolates in this survey were tested for

antibiotic susceptibility and resistance in their planktonic form and these results may fluctuate in vivo. Despite these limitations, no correlation for antibiotic susceptibility or resistance was determined for tube duration, location of the isolate on the feeding tube, and dietary supplements administered through the gastrostomy device.

Instead of distinguishing colonization from infection, our initial work focused on identifying the types of bacterial species associated with pediatric feeding tubes. Numerous isolates characterized in that study are potential pathogens that have been associated with opportunistic infections in immunocompromised patients.²⁶ This work further supports these initial findings, indicated by the extensive single and multiple antibiotic resistances determined for the 100 isolates. Due to the lack of antibiotic resistance surveillance studies focusing on bacteria colonizing pediatric feeding tubes, our results will provide pediatricians with a starting point for antibiotic selection against possible secondary infections resulting from surgically implanted gastrostomy tubes.

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REFERENCES

1. Suci PA, Vransky JD, Mittelman MW. Investigation of interactions between antimicrobial agents and bacterial biofilms using

- attenuated total reflection Fourier transform infrared spectroscopy. *Biomaterials*. 1988;19:327-339.
2. Olson ME, Ceri H, Morck DW, et al. Biofilm bacteria: formation and comparative susceptibility to antibiotics. *Can J Vet Res*. 2002;66:86-92.
 3. Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. *Int J Med Microbiol*. 2002;292:107-113.
 4. Hoyle BD, Jass J, Costerton JW. The biofilm glycocalyx as a resistance factor. *J Antimicrob Chemother*. 1990;26:1-5.
 5. Costerton JW, Lewandowski Z. The biofilm lifestyle. *Adv Dent Res*. 1997;11:192-195.
 6. Monzón M, Oteiza C, Leiva J, et al. Synergy of different antibiotic combinations in biofilms of *Staphylococcus epidermidis*. *J Antimicrob Chemother*. 2001;48:793-801.
 7. Gander S, Hayward K, Finch R. An investigation of the antimicrobial effects of linezolid on bacterial biofilms utilizing an in vitro pharmacokinetic model. *J Antimicrob Chemother*. 2002;49:301-308.
 8. Xu KD, McFeters GA, Stewart PS. Biofilm resistance to antimicrobial agents. *Microbiol*. 2000;146:547-549.
 9. DeLancey Pulcini E. Bacterial biofilms: a review of current research. *Nephrologie* 2001;22:439-441.
 10. Stephens C. Microbiology: breaking down biofilms. *Curr Biol*. 2002;12:R132-134.
 11. Stewart PS. Biofilm accumulation model that predicts antibiotic resistance of *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother*. 1994;38:1052-1058.
 12. Gilbert P, Brown MWR. Mechanisms of the protection of bacterial biofilms from antimicrobial agents. In: Lappin-Scott HM, Costerton JW, eds. *Microbial Biofilms*. Cambridge, England: Cambridge University Press; 1995:118-130.
 13. Adam D. Global antibiotic resistance in *Streptococcus pneumoniae*. *J Antimicrob Chemother*. 2002;50:1-5.
 14. El-Adhami W. Expression of a clone specific DNA sequence from *Staphylococcus aureus* in *Escherichia coli*. *J Biotechnol*. 1999;73:181-184.
 15. Dautle MP, Ulrich RL, Hughes TA. Typing and sub-typing of 83 clinical isolates purified from surgically implanted silicone feeding tubes by random amplified polymorphic DNA amplification. *J Clin Microbiol*. 2002;40:414-421.
 16. National Committee for Clinical Laboratory Standards: Performance Standards for Antimicrobial Disk Susceptibility Tests – Seventh Edition: Approved Standard M2-A7. NCCLS, Wayne, PA, 2000
 17. National Committee for Clinical Laboratory Standards: Performance Standards for Antimicrobial Susceptibility Testing – Tenth Edition: Informational Supplement M100-S10(M2). Wayne, PA: NCCLS; 2000.
 18. Wiggins BA. Discriminant analysis of antibiotic resistance patterns in fecal streptococci, a method to differentiate human and animal sources of fecal pollution in natural waters. *Appl Environ Microbiol*. 1996;62:3997-4002.
 19. DuBois SK, Marriott MS, Amyes SGB. TEM- and SHV-derived extended-spectrum β -lactamases: relationship between selection, structure, and function. *J Antimicrob Chemother*. 1995;35:7-22.
 20. Ghigo JM. Natural conjugative plasmids induce bacterial biofilm development. *Nature*. 2001;412:442-445.
 21. Hogan D, Kolter R. Why are bacteria refractory to antimicrobials? *Curr Opin Microbiol*. 2002;5:472-477.
 22. Roberts AP, Cheah G, Ready D, et al. Transfer of Tn916-like elements in microcosm dental plaques. *Antimicrob Agents Chemother*. 2001;45:2943-2946.
 23. Wang BY, Chi B, Kuramitsu HK. Genetic exchange between *Treponema denticola* and *Streptococcus gordonii* in biofilms. *Oral Microbiol Immunol*. 2002;17:108-112.
 24. Barry AL, Jones RN. In vitro activity of ciprofloxacin against gram-positive cocci. *Am J Med*. 1987;82(suppl 4A):27-32,
 25. Pien ECT, Hume KE, Pien FD. Gastrostomy tube infections in a community hospital. *Am J Infect Control*. 1996;24:353-358.
 26. Dautle MP, Wilkinson TR, Gauderer MWL. Isolation and identification of biofilm microorganisms from silicone gastrostomy devices. *J Pediatr Surg*. 2003;38:216-220.