Assessment of Microbiota and Their Drug Resistance in Chronic Fistulous Tracts

Xiuwen Wu,¹ Jianan Ren,¹ Xiaoting Li,² Song Liu,¹ Qin Wu,¹ Gefei Wang,¹
Guosheng Gu,¹ Huajian Ren,¹ and Jieshou Li¹

Abstract

Background: Bacteria colonizing an enterocutaneous fistula tract have not been clarified. The aims of this study were to investigate the pathogen spectra of fistulous tracts and their resistance to antibiotics in patients with chronic fistulas.

Methods: We conducted a one-year prospective single-center study. In the absence of significant sepsis, consecutively stabilized patients with chronic enterocutaneous fistula were included. Microbiology and antimicrobial susceptibility of isolates from the tracts were analyzed. The correlations between the existence of bacteria and various clinical values were investigated further.

Results: Forty-one patients were enrolled, of whom eight had a negative culture. A total of 48 bacterial strains were harvested, including 42 strains of gram-negative bacteria and six strains of gram-positive bacteria, most of which were multiple-drug-resistant. The three bacteria cultured most often were *Escherichia coli* (11 strains; 22.9%), *Pseudomonas aeruginosa* (eight strains; 16.7%), and *Klebsiella pneumoniae* (eight strains). Binary logistic regression analysis with forward (conditional) stepwise selection found that fistula length correlated with positive bacterial results ($p=0.018$). Other variables, namely entire length of hospitalization and fistula duration and location, were unrelated to the presence of micro-organisms in fistula tracts.

Conclusions: Multiple-drug-resistant gram-negative bacteria were the main pathogens colonizing chronic fistula tracts. Fistula length was significantly associated with the presence of pathogens in a multivariable logistic regression model.

MICROBIOLOGIC PROFILES and antimicrobial susceptibility in patients with enterocutaneous fistula (ECF) associated with intra-abdominal infections has been investigated [1]. However, bacteria colonizing the fistulous tracts have not been clarified. The present study was undertaken to assess the bacteriologic spectrum in patients with chronic ECFs and to investigate anti-microbial resistance profiles. A further aim was to identify the potential risk factors for the presence of pathogens in the chronic tracts.

Patients and Methods

Study population

From December 2011 to December 2012, we collected prospectively the clinical records as well as pathogens colonizing fistulous tracts from patients with chronic ECFs at the

Department of Surgery, Jinling Hospital. Stabilized patients with a single tubular (tract length >2 cm) fistula, low output volume (<200 mL/24 h), and chronic and persistent fistula >3 mo were enrolled. Exclusion criteria were as follows: Cancer-infiltrated fistula, associated abscess, and any signs of infection. The entire study protocol was approved by the Institutional Review Board Ethics Committee of Jinling Hospital, and all research work with human beings was in compliance with the Helsinki Declaration.

Conventional microbiology

Samples from the secretions of fistula tracts were collected after saline rinsing. An aliquot of secretion was obtained with a sterile cotton swab (Zhejiang Gongdong Medical Technology Co. Ltd, Taizhou, Zhejiang, China) and transferred to a culture tube. Aerobic bacterial cultures were made according

¹Department of Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing, China.

²Nanjing University of Chinese Medicine, Nanjing, China.

*Presented at the Thirty-third Annual Meeting of the Surgical Infection Society, Las Vegas, Nevada, April 12–15, 2013.

to standard laboratory methods [2]. The samples were immersed in liquid growth medium (aerobic blood culture bottles) and subjected to standard culture using various media including blood, McConkey, and Sabouraud agar plates in a routine manner by a certified academic microbiology laboratory. After incubation, the medium was examined by an experienced clinical microbiologist, and pure cultures were identified. For such cultures, the strains involved were stored for future use. For mixed cultures, the biomaterial was collected from the plate and stored in glycerol-containing medium. After presumptive identification, pure cultures were subjected to precise species identification using Vitek technology and the API identification system (BioMérieux, Hazelwood, MO).

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of the following 19 antimicrobial agents were determined by the agar dilution method according to the Clinical Laboratory Standards Institute (CLSI) guidelines [3]: Ampicillin (AMP), piperacillin (PIP), cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), ceftiofloxacin (FOX), aztreonam (ATM), nalidixic acid (NAL), ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR), gatifloxacin (GAT), gentamicin (GM), amikacin (AMK), chloramphenicol (CHL), trimethoprim/sulfamethoxazole (SXT), tetracycline (TET), and imipenem-cilastatin (IMP). Multi-drug resistance (MDR) was defined as resistance to three or more of the above antibiotic classes. *Staphylococcus aureus* American Type Culture Collection (ATCC) 25923 (American Type Culture Collection, Rockville, MD), *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control. Susceptible and non-susceptible isolates were defined according to the criteria used for Enterobacteriaceae by the CLSI [3].

Data retrieval

The medical records were reviewed, with collection of demographics (age, gender, residence), co-morbid conditions (diabetes mellitus, congestive heart failure, chronic obstructive pulmonary disease, chronic kidney disease, hepatic

disease, malignant disease), main indication for hospitalization, and fistula characteristics (i.e., length and width, duration, output, location). Information on procedures prior to and after the positive secretion culture, such as placement of a central venous catheter, admission to the intensive care unit (ICU), hemodialysis, invasive procedures (cardiovascular and endovascular catheterization, endoscopic procedures, tracheostomy), surgery, and mechanical ventilation was retrieved. The antibiotic therapy during hospitalization and classes of antibiotics were collected also.

Statistical analysis

In univariate analysis, both the unpaired *t*-test and the χ^2 test were used to report any difference between groups with positive or negative microbial results. Multivariable logistic regression was performed to identify factors that influenced independently the presence of pathogens inside the tracts. Variables in univariate analysis were chosen to enter the multivariable analysis if $p < 0.05$. All statistical analyses were done with SPSS software (IBM, Inc., Armonk, NY). All tests were two-tailed, and a p value < 0.05 was considered to be statistically significant.

Results

Microbial flora analysis

During the study period, 41 patients with chronic fistulas were enrolled, and secretions from fistula tracts were collected for microbiologic analysis (Fig. 1). Tract culture showed growth in 33 fistulas. A total of 48 bacterial strains was harvested, including 42 of gram-negative and six of gram-positive bacteria. Microbial species were bowel derived, skin derived, or a combination. The three most frequently isolated bacteria were *E. coli* (11 strains; 22.9%), *Klebsiella pneumoniae* (eight strains; 16.7%), and *P. aeruginosa* (eight strains; 16.7%).

Antimicrobial resistance

Comparative analyses of the sensitivities of the three most common isolates to the individual antibiotics were

FIG. 1. Distribution of organisms from fistula tracts.

TABLE 1. ANTIMICROBIAL CHARACTERISTIC OF PATHOGENS

Antimicrobial agent or characteristic	Breakpoint (mcg/mL)	No. of resistant isolates (%)		
		<i>Escherichia coli</i> (n = 11)	<i>Klebsiella pneumoniae</i> (n = 8)	<i>Pseudomonas aeruginosa</i> (n = 8)
ESBL-producing		5 (45.5)	2 (25.0)	—
AMK	≥ 64	1 (9.1)	2 (25.0)	1 (12.5)
ATM	≥ 64	9 (81.8)	5 (62.5)	5 (62.5)
AMP	≥ 32	11 (100)	8 (100)	8 (100)
SAM	≥ 32	11 (100)	6 (75.0)	8 (100)
ETP	≥ 8	1 (9.1)	3 (37.5)	—
SXT	≥ 320	9 (81.8)	4 (50.0)	8 (100)
CIP	≥ 4	10 (90.9)	7 (87.5)	3 (37.5)
GM	≥ 16	9 (81.8)	4 (50.0)	3 (37.5)
CRO	≥ 64	10 (90.9)	7 (87.5)	8 (100)
CAZ	≥ 64	9 (81.8)	—	1 (12.5)
FOX	≥ 64	3 (27.3)	4 (50.0)	8 (100)
FEP	≥ 64	7 (63.6)	6 (75.0)	2 (25.0)
KZ	≥ 64	10 (90.9)	7 (87.5)	8 (100)
CTX	—	9 (81.8)	7 (87.5)	4 (50.0)
TOB	≥ 16	4 (36.4)	4 (50.0)	3 (37.5)
IMP	≥ 16	2 (18.2)	4 (50.0)	5 (62.5)
LEV	≥ 8	10 (90.9)	5 (62.5)	3 (37.5)
F	≥ 512	—	2 (25.0)	4 (50.0)
TZP	≥ 128	3 (27.3)	4 (50.0)	—
Multi-drug-resistant ^a	—	11 (100)	7 (87.5)	8 (100)

^aResistant to three or more antimicrobial agents.

AMK = amikacin; ATM = aztreonam; AMP = ampicillin; AMS = ampicillin-sulbactam; CAZ = ceftazidime; CIP = ciprofloxacin; CRO = ceftriaxone; CTX = ceftazolin; ETP = ertapenem; ESBL = extended-spectrum β -lactamase; FEP = cefepime; FOX = ceftazidime; GM = gentamicin; IMP = imipenem-cilastatin; KZ = cefazolin; LEV = levofloxacin; SXT = trimethoprim-sulfamethoxazole; TOB = tobramycin.

performed, as shown in Table 1. Overall, among the 27 gram-negative isolates, 26 (96.3%) were MDR. All strains of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were resistant to AMP, and 100% of the *E. coli* and *P. aeruginosa* strains were resistant to SAM, compared with 75.0% of *K. pneumoniae*. Further, all *P. aeruginosa* strains, 90.9% of the *E. coli* strains, and 87.5% of the *K. pneumoniae* strains were resistant to CRO and KZ.

Production of extended-spectrum β -lactamase (ESBL)

Nearly 40% (7/19) of the Enterobacteriaceae were Extended-Spectrum β -Lactamase (ESBL) producers (Table 1). The highest producer rate was documented in *E. coli* (5/11), compared with only 25.0% (2/8) of *K. pneumoniae*. The impact of ESBL production on susceptibility rates to antibiotic classes was as follows: Resistance rates to ATM and CTX by ESBL-producing *E. coli* and *K. pneumoniae* were 100%.

Clinical characteristics

Thirty-three men and eight females with a mean age of 47 ± 15.0 y (range 18–75 y) were investigated. Patient demographics, co-morbidities, underlying diseases, medication, and other risk factors during hospital stay are shown in Table 2 and Table 3. There were no significant differences among those fistulas with or without colonizing pathogens according to demographics, co-morbidities, medication, underlying diseases, and other risk factors. The only significant difference lay in the entire length of stay (LOS), which was remarkably longer in the bacteria-positive group than that in

the negative group ($p=0.0063$). Among fistula variables, duration, site, and length were significantly different in the two groups ($p=0.0440$, $p=0.0218$, and $p=0.0492$, respectively) (Table 4).

Univariate analysis

Univariable analysis (χ^2 test for nominal and t -test for interval variables) was used to find the relation between potential risk factors and positive culture results. Significantly longer hospital stays were observed in the positive culture group (see Table 2). Lower gastrointestinal

TABLE 2. BASELINE CHARACTERISTICS OF PARTICIPANTS

Variable	Negative (n = 8)	Positive (n = 33)	p
Mean age (standard deviation)	41 (13.4)	41 (7.5)	0.2464
Male:female	7:1	30:3	1.0000
Mean body mass index (standard deviation)	19.9 (1.75)	19.4 (0.41)	0.6895
Co-morbidities (%)			
Anemia	2 (25.0)	17 (51.5)	0.2488
Malignant disease	1 (12.5)	5 (15.2)	1.0000
Hypertension	2 (25.0)	6 (18.2)	0.6423
Diabetes mellitus	0	5 (15.2)	0.5632
Coronary artery disease	1 (12.5)	1 (3.0)	0.3561
Smoking	2 (25.0)	10 (30.3)	1.0000
Alcohol abuse	2 (25.0)	12 (36.4)	0.6925

TABLE 3. UNIVARIABLE ANALYSIS OF RISK FACTORS

	Negative (n=8)	Positive (n=33)	p
Mean LOS ^a (SD)	104 (7.5)	127 (23.2)	0.1258
Mean entire LOS ^b (SD)	136 (17.1)	206 (10.2)	0.0063
Previous antibiotics (%)			
Carbapenem in combination therapy	3 (37.5)	11 (33.3)	1
CAZ or FEP in combination therapy	3 (37.5)	16 (48.5)	0.7033
TZP in combination therapy	2 (25.0)	3 (9.1)	0.2465
VAN in combination therapy	1 (12.5)	7 (21.2)	1
Antifungal therapy	3 (37.5)	13 (39.4)	1
Central venous catheter (%)	7 (87.5)	25 (75.8)	0.6586
Mechanical ventilation (%)	2 (25.0)	6 (18.2)	0.6423
Surgery (%)	8 (100)	33 (100)	–
Invasive procedure (%)	3 (37.5)	14 (42.4)	1
Endoscopy (%)	2 (25.0)	3 (9.1)	0.2465
Admission to ICU (%)	2 (25.0)	7 (21.2)	1
Mean LOS in ICU (SD)	12 (4.2)	8 (2.8)	0.1482

^aDays since admitted to our hospital.^bDays since first hospitalization.

CAZ=ceftazidime; FEP=cefepime; ICU=intensive care unit; LOS=length of stay; SD=standard deviation; TZP=piperacillin-tazobactam; VAN=vancomycin.

(GI) fistulas, longer tracts (>5 cm), and longer duration of fistulization were more prevalent in the positive group (Table 4).

Multivariable analysis

In a multivariable logistic regression model, adjusted by entire LOS, fistula duration, and location, tract length was

TABLE 4. UNIVARIATE ANALYSIS OF FISTULA FACTORS RELATED TO BACTERIAL CULTIVATION

	Negative (n=8)	Positive (n=33)	p
Median duration (SD)	160 (79.1)	195 (105.8)	0.0440
Median output (SD)	78 (50.1)	146 (94.2)	0.4861
Location (%)			0.0218
Upper gastrointestinal	5 (62.5)	6 (18.2)	
Lower gastrointestinal	3 (37.5)	27 (81.8)	
Length (cm) (%)			0.0492
<5	7 (87.5)	11 (33.3)	
5–9	1 (12.5)	13 (39.4)	
10–14	0	7 (21.2)	
15–20	0	2 (6.1)	
Width (cm) (%)			0.3122
<0.4	3 (37.5)	7 (21.2)	
0.5–0.7	3 (37.5)	22 (66.7)	
0.8–1.0	2 (25.0)	4 (12.1)	

SD=standard deviation.

TABLE 5. MULTIVARIABLE ANALYSIS OF RISK FACTORS FOR PATHOGENS

	Odds ratio	95% Confidence Interval	p
Entire length of stay	2.109	0.343–12.986	0.421
Fistula duration	1.160	0.231– 5.814	0.857
Fistula location	4.169	0.581–29.905	0.156
Fistula length	8.832	1.936–83.340	0.018

significantly associated with the presence of pathogens inside the tracts ($p=0.018$) (Table 5). The Hosmer-Lemeshow test showed a goodness of fit p for this logistic regression model of 0.997. The c-statistic index (receiver-operating characteristic (ROC) curve) was 0.771 ($p=0.019$, 95% confidence interval 0.616–0.926). The Durbin Watson statistic showed no problems with serial autocorrelation ($D-W=2.112$).

Discussion

In our study, gram-negative micro-organisms dominated the fistula tracts, and the top three isolated bacteria most commonly were *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. The susceptibilities of these isolates to the individual antibiotics demonstrated that 96.3% (26/27) of them were MDR. Longer tracts (>5 cm) were an independent risk factor for the presence of pathogens inside the fistula tracts in a multivariable regression model, adjusted by entire hospital stay.

The pathogenicity of the organisms found in this study should attract much attention. It might be attributable to the combination of intensive use of antibiotics, long hospitalization, and cross-contamination. Many people believe that because antibiotics are a natural product of bacteria, the resistance mechanisms are not new. However, the evolution of present epidemics of resistant healthcare-associated infections has been, to some extent, antibiotic-driven [4]. The evidence points to unnecessary or inappropriate antibiotic therapy in hospitals, much of it not consistent with clinical guidelines [5]. On the other hand, MDR pathogens can survive for a long time in the hospital environment and can be transferred easily between patients through the hands of healthcare workers [6, 7]. What is worse, it is no longer a phenomenon restricted to the healthcare setting. Emergence of community-acquired antibiotic resistance, such as *E. coli* producing CTX-M extended-spectrum β -lactamases and fluoroquinolones have been reported increasingly in the U.S., Europe, and Asia [8, 9].

The antibiotic resistance nowadays needs global responses. National approaches and commitment would be effective in controlling resistance [10, 11]. The cornerstone of reducing resistance is practicing high-quality antimicrobial stewardship and infection control. Essential antibiotic stewardship in the hospital and the community includes avoiding unnecessary antimicrobial exposure, distinguishing colonization from true infection, dosing antimicrobials adequately, and stopping antimicrobial treatment as soon as possible after symptom resolution [12].

Better than treatment of infection is, of course, prevention. But prevention of health-care-associated infections at the hospital level is challenging. Besides hand hygiene, the

importance of contact precautions in all healthcare settings cannot be overemphasized.

In conclusion, this study found that MDR gram-negative bacteria were the main pathogens inside fistulous tracts. Fistula length was significantly associated with the presence of pathogens inside the tracts in a multivariable logistic regression model.

Acknowledgment

This work was supported by grants from the National Natural Science Foundation of China (81270478).

Author Disclosure Statement

None of the authors has any conflict of interest to declare concerning the manuscript.

References

1. Zhou Z, Ren J, Wang G, et al. Analysis of pathogen spectra and their drug resistance in patients with enterocutaneous fistula complicated with abdominal infection. *Chin J Dig Surg* 2008;7:331–333.
2. Balows A, Hausler WJ Jr, Hermann KL, et al. *Manual of Clinical Microbiology*, Fifth Edition. Washington, DC: American Society for Microbiology, 1991.
3. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-Second Informational Supplement, M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute. 2012.
4. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance—the need for global solutions. *Lancet Infect Dis* 2013;13:1057–1098.
5. Davey P, Brown E, Charani E, et al. Interventions to improve antibiotic prescribing practices for hospital inpatients. *Cochrane Database Syst Rev* 2013;4:CD003543.
6. Holden MT, Hsu LY, Kurt K, et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. *Genome Res* 2013;23:653–664.
7. Ammerlaan HS, Harbarth S, Buiting AG, et al. Secular trends in nosocomial bloodstream infections: Antibiotic-resistant bacteria increase the total burden of infection. *Clin Infect Dis* 2013;56:798–805.
8. Zaidi AK, Huskins WC, Thaver D, et al. Hospital-acquired neonatal infections in developing countries. *Lancet* 2005;365:1175–1188.
9. Waters D, Jawad I, Ahmad A, et al. Aetiology of community-acquired neonatal sepsis in low and middle income countries. *J Glob Health* 2011;1:154–170.
10. Stone SP, Fuller C, Savage J, et al. Evaluation of the National Clean Your Hands Campaign to reduce *Staphylococcus aureus* bacteraemia and *Clostridium difficile* infection in hospitals in England and Wales by improved hand hygiene: Four year, prospective, ecological, interrupted time series study. *BMJ* 2012;344:e3005.
11. Schwaber MJ, Lev B, Israeli A, et al, for the Israel Carbapenem-Resistant Enterobacteriaceae Working Group. Containment of a country-wide outbreak of carbapenem-resistant *Klebsiella pneumoniae* in Israeli hospitals via a nationally implemented intervention. *Clin Infect Dis* 2011; 52: 848–855.
12. Pop-Vicas A, Opal SM. The clinical impact of multidrug-resistant gram-negative bacilli in the management of septic shock. *Virulence* 2013;5.

Address correspondence to:

Dr. Jianan Ren

Department of Surgery

Jinling Hospital

Medical School of Nanjing University

305 East Zhongshan Rd.

Nanjing, 210002, China

E-mail: JiananR@gmail.com