Analysis of Predictive Factors of Antibiotic Resistant *Pseudomonas aeruginosa* in Children with Acute Appendicitis

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Abstract

Objectives: To analyze the antibiotic resistance in children with acute appendicitis caused by *Pseudomonas aeruginosa (P. aeruginosa)*, and to explore the predictors of infection and antibiotic resistance in children with acute appendicitis through blood biochemical indicators

Methods: Fifty control children (with no bacteria detected in the culture) and 103 children with *P. aeruginosa* infection who were admitted to the general surgery department of Jiangxi children's hospital and treated surgically from January 2015 to June 2019 were analyzed.

Results: Cefotaxime, cotrimoxazole and tetracycline had the highest drug resistance rates in children with appendicitis caused by *P. aeruginosa*, with (93.20%, 96/103), (93.20%, 96/103) and (93.20%, 96/103), respectively. There were significant pattern differences between the *P. aeruginosa* infected group and penicillin antibiotics resistant group. The combination of glutamyltranspeptidase (GGT), phosphorus (P), free fatty acid (FFA) and fibrinogen (CFbg) could effectively identify whether children with acute appendicitis were infected by *P. aeruginosa* and whether they were resistant to penicillin antibiotics. For the control group and *P. aeruginosa* infected group, area under the receiver operating characteristic (AUROC) value of the four indicators was 0.9034 (95% CI, 0.8514 - 0.9555), with a sensitivity and specificity of 84.76% and 87.93%, respectively. For the group with or without penicillin antibiotics resistance, the AUROC value was 0.9341 (95% CI, 0.8681 - 1.0000), with a sensitivity and specificity of 89.04% and 96.88%, respectively.

Conclusions: Clinical blood biochemical indicators can reflect bacterial infection and antibiotic resistance in children with acute appendicitis to a certain extent, and the combination of GGT, P, FFA, and CFbg can predict *P. aeruginosa* infection and penicillin antibiotics resistance in children with acute appendicitis.

Keywords: Analysis, *Pseudomonas aeruginosa*, Antibiotic, Appendicitis

1. Background

Acute appendicitis is a common acute abdominal disease in pediatric surgery (1), and delayed diagnosis and treatment can easily lead to complications such as appendix perforation and sepsis (2, 3). The pathogenesis of the disease is still not fully understood, and obstruction of the appendiceal cavity and bacterial infection are considered to be the main causes of the disease (4), among which *Escherichia coli* and *Pseudomonas aeruginosa* are the common pathogens (5, 6). Due to the particularity of pediatric patients, the diversity of antibacterial drugs and the increase of multi-drug resistance of bacteria, the antibiotic treatment program for acute appendicitis in children becomes increasingly complex (7, 8). Therefore, the understanding of pathogenic bacteria and the drug resistance spectrumis is conducive to the optimization of the empirical antimicrobial treatment program and the improvement of the clinical treatment effect, can also reduce the use of unnecessary antimicrobial agents.

Studies have found that the number of *P. aeruginosa* isolated from the secretion of the appendix cavity of patients with acute appendicitis is positively correlated to the infection degree of the operation site, and the reason may be related to the lack of corresponding antibiotics in the primary treatment scheme (9, 10). Although imaging techniques (11, 12), inflammatory factors (13-17), some clinical biochemical indicators (18, 19) and some blood metabolites (20) have been able to diagnose acute appendicitis,

the pathogenic bacteria and drug resistance spectrum in patients have not been determined by non-interventional methods.

2. Objectives

This study intends to establish a suitable non-interventional differential diagnosis model to determine whether the pathogenic bacteria in children are *P. aeruginosa* and if so, to determine its antibiotic resistance through statistical analysis of clinical blood biochemical indicators of children with acute appendicitis, and to screen out appropriate predictors.

3. Methods

3.1. Study Population

This retrospective study was approved by the Ethics Committee of Jiangxi Provincial Children's Hospital (code: No.JXSETYY-YXKY-20190096). In this study, patients with acute appendicitis admitted to the general surgery department of Jiangxi children's hospital from January 2015 to June 2019 and treated surgically were enrolled. A total of 153 patients were included in this study, aged 1 - 16 years, 99 males and 54 females. All samples were identified and tested by BD Phoenix, a microbial identification and drug sensitivity analysis system. The subjects' appendiceal secretions or pus were taken for bacterial culture, among which 50 cases (group of control) of bacterial infection were not detected, and 103 cases of *P. aeruginosa* infection were detected. According to the resistance of *P. aeruginosa* to penicillin antibiotics, 72 cases were classified as drug resistant group (PAR+) and 31 cases as non-drug resistant group (PAR-). The clinical blood test parameters of all subjects are shown in Supplementary table 1 and the clinical information are summarized in Table 1.

3.2. Statistical Analysis

All the data in this study were based on 71 clinical blood test indexes recorded in the original medical records of the subjects at admission. All the data were imported into SIMCA-P (version 12.0, Umetrics, Umea, Sweden) for partial lead squares discrimination analysis (PLS-DA) and SPSS23.0 (SPSS Inc., Chicago, IL, US) for Mann Whitney U-test, ROC analysis and odds ratio (OR) calculation.

4. Results

4.1. Analysis of Antibiotic Resistance of Pseudomonas aeruginosa in Abdominal Cavity of Children with Acute Appendicitis

Bacterial culture and antibiotic resistance experiments were conducted on exudates or pus in the appendiceal cavity of all subjects. A total of 103 strains of *P. aeruginosa* were isolated and showed resistance to 14 antibiotics (Table 2). Among them, 4 strains were sensitive to all types of antibiotics, 3 strains showed resistance to only one antibiotic, and 96 showed resistance to three or more antibiotics. Beta lactam antibiotic is the most common type of the 14 antibiotics (7/14), and 99 strains of *P. aeruginosa* showed resistance to beta-lactam antibiotic, with the resistance rate of 100.00% (99/99). The three drugs with the highest rate of individual antibiotic resistance were cefotaxime (93.20%, 96/103), cotrimoxazole (93.20%, 96/103), and tetracycline (93.20%, 96/103).

4.2. Analysis Results of Blood Indicators of all Subjects and the Establishment of Discriminant Model

Subject data were imported into SIMCA-P for multivariate statistical analysis, and the results are shown in Figure 1A and B. Control/*P. averuginosa* and PAR+/PAR- were distributed in different areas of the figure, indicating significant pattern differences between Control/*P. averuginosa* and PAR+/PAR- (21). Control/*P. averuginosa* and PAR+/PAR- showed significant differences in 33 and 20 variables, respectively (Appendix 1 in Supplementary File). Among them, 7 variables (ALB, IDBL, GGT, CRP, P, FFA, and CFbg) showed significant differences among each group (Table 3).

To establish a suitable differential diagnosis model, the above seven variables were used as parameters for binary logistic regression analysis, and the maximum likelihood estimation forward method (forward Wald) was used for stepwise regression analysis.

The results showed that control/*P. aeruginosa* and PAR+/PAR- could be effectively distinguished by combining GGT, P, FFA, and CFbg. For the control group and *P. aeruginosa* infected group, the AUROC value of the four indicators was 0.9034 (95% CI, 0.8514 - 0.9555), with a sensitivity and specificity of 84.76% and 87.93%, respectively (Figure 1C). For the group with or without penicillin antibiotics resistance, the AUROC value was 0.9341 (95% CI, 0.8681 - 1.0000), with a sensitivity and specificity of 89.04% and 96.88%, respectively (Figure 1D).

4.3. Predictive Analysis of Pseudomonas aeruginosa Infection and Drug Resistance in Children with Acute Appendicitis

Multiple logistic regression was used to analyze GGT, P, FFA, and CFbg variables, and the OR value of a single

Table 1. Clinical Information for 153 Patients

Group	Total Cases	Male	Female	Age, y	Hospital Stay, d	Appendix Perforated
Group of control	50	35	15	6.51 ± 0.39	7.45 ± 0.33	2
Pseudomonas aeruginosa (PRA+)	72	46	16	6.80 ± 0.37	9.47 ± 0.37	60
P. aeruginosa (PRA-)	31	24	7	6.90 ± 0.53	9.83 ± 0.59	24

Table 2. Antibiotic Resistance in Children with Appendicitis Caused by *Pseudomonas aeruginosa*

No	Antibiotics	*Pseudomonas aeruginosa* (N=103)		Total	Ratio of Resistance, %
		PAR- (N = 31)	PAR+ (N = 72)		
1	Ampicillin	0	69	69	66.99
2	Piperacillin	0	3	3	2.91
3	Cefepime	0	2	2	1.94
4	Cefotaxime	24	72	96	93.20
5	Ceftazidime	0	2	2	1.94
6	Cefazolin	22	70	92	89.32
7	Aztreonam	2	7	9	8.74
8	Cotrimoxazole	24	72	96	93.20
9	Tetracycline	24	72	96	93.20
10	Chloramghenicol	23	72	95	92.23
11	Gentamicin	2	1	3	2.91
12	Levofloxacin	0	4	4	3.88
13	Ciprofloxacin	1	4	5	4.85
14	Amikacin	0	1	1	0.97

Table 3. Seven Variables with Significant Difference in Control/*Pseudomonas aeruginosa* and PAR+/PAR-[a]

Factors	Control	*Pseudomonas aeruginosa*		P1	P2
		PAR-	PAR+		
Num.	50	31	72	-	-
Male	30	24	45	3.54E-01	1.65E-01
Age, y	6.51 ± 0.39	6.9 ± 0.53	6.80 ± 0.37	7.28E-02	5.99E-01
ALB, g/L	47.42 ± 0.42	45.43 ± 0.77	44.11 ± 0.42	8.97E-06	4.57E-02
IDBL, μmol/L	6.72 ± 0.34	10.18 ± 0.87	8.26 ± 0.47	2.35E-03	3.34E-02
CRP, mg/L	18.56 ± 3.58	67.31 ± 11.16	93.80 ± 7.87	1.45E-10	3.15E-02
GGT, U/L	8.52 ± 0.78	8.94 ± 0.96	12.04 ± 0.63	6.13E-05	4.00E-04
P, mmol/L	1.61 ± 0.04	1.39 ± 0.08	1.37 ± 0.06	2.37E-07	3.32E-05
FFA, mmol/L	0.90 ± 0.06	0.87 ± 0.09	1.22 ± 0.08	2.97E-04	3.35E-13
CFbg, g/L	2.79 ± 0.08	3.98 ± 0.22	4.53 ± 0.19	2.92E-12	3.31E-02.

Abbreviations: ALB, albumin; CRP, C-reactive protein; CFbg, fibrinogen; FFA, free fatty acid; GGT, glutamyltranspeptidase; IDBL, indirect bilirubin; P, phosphorus; P1, P-value of Control vs. *P. aeruginosa*; P2, P-value of PAR- vs. PAR+.
[a] Values are expressed as mean ± SEM.

variable and the combination of the four variables were calculated respectively (Table 4). The results showed that the effect of the combination of four variables is signifi-

cantly better than that of a single variable, and the OR value of control/*P. aeruginosa* and PAR+/PAR- were 655.9676 (95% CI, 109.9204 - 3914.5928) and 1115.8482 (95% CI, 121.2366 -
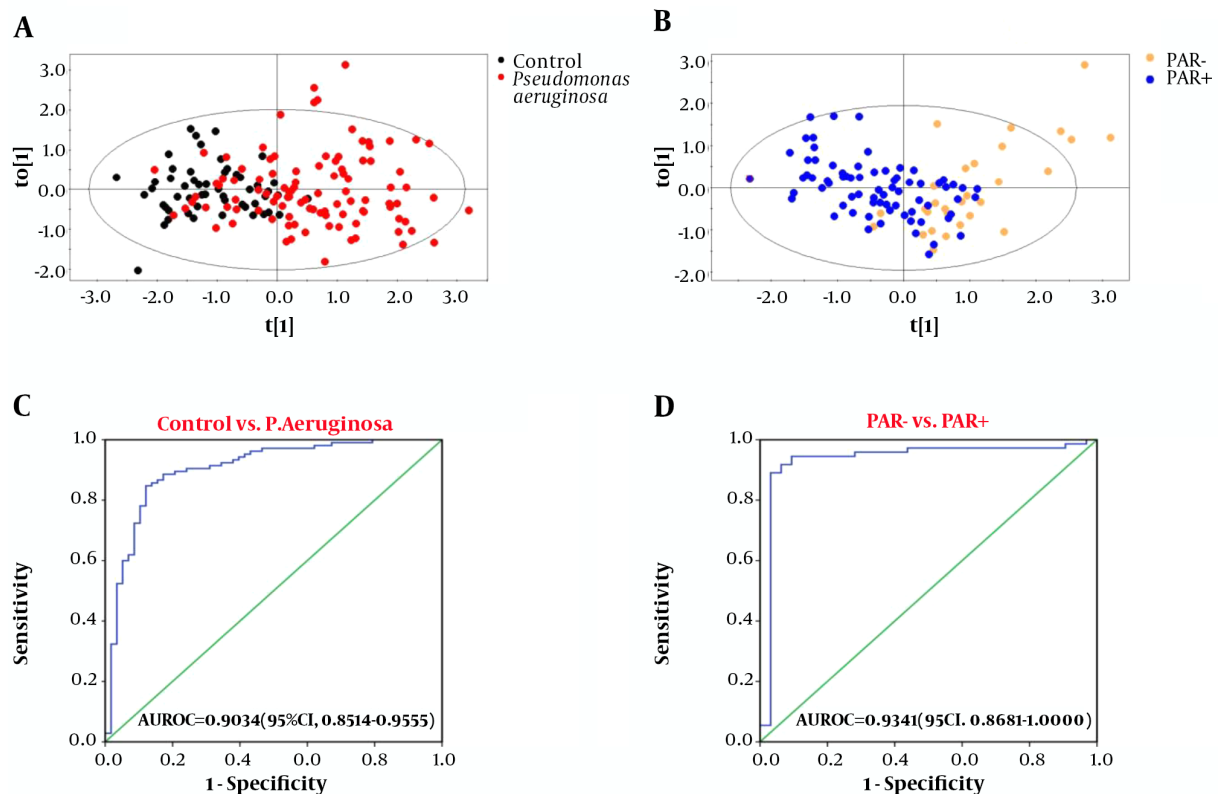
Figure 1. Results of multivariate statistical analysis. A, PLS-DA score of control/*Pseudomonas aeruginosa*; B, PLS-DA score of PAR+/PAR-; C, ROC analysis score of control/*P. aeruginosa*; D, ROC analysis score of PAR+/PAR-.

10270.1414), respectively. It suggests that the combination of GGT, P, FFA, and CFbg can be used to judge *P. aeruginosa* infection and penicillin antibiotics resistance in children with appendicitis to a certain extent.

5. Discussion

Bacterial infection and antibiotic resistance in patients with appendicitis are a difficult problem in today's world, and how to develop effective treatment plans early is a challenge for clinicians ([22]). It is of great significance to understand the pathogenic bacteria and drug resistance spectrum of patients with appendicitis and use drugs in a targeted way to improve the clinical treatment effect, reduce the abuse of antibiotics and reduce the drug resistance rate of antibiotics. In this study, 103 children with acute appendicitis infected by *P. aeruginosa* were analyzed. It was found that the drug resistance rate of pathogenic bacteria to antibiotics was very high (96.12%, 99/103). The drug resistance rate of resistant bacteria to beta-lactam antibiotic reached 100.00% (99/99), and 96.97% (96/99) of resis-

tant bacteria showed multiple drug resistance. Therefore, we should reduce the use of cefotaxime (93.20%), cefazolin (89.32%), cotrimoxazole (93.20%), tetracycline (93.20%) and chloromeganicol (92.23%) and other antibiotics with high drug resistance rate, and use highly sensitive antimicrobials such as cefepime (1.94%) and ceftazidime (1.94%).

In this study, for the first time, the pattern recognition analysis was conducted on the clinical indicators of acute appendicitis in children with negative bacterial culture and *P. aeruginosa* infection through multivariate statistical analysis, and found that the combination of GGT, P, FFA and CFbg could effectively distinguish control/*P. aeruginosa* and PAR+/PAR-. Compared with the control group, the contents of GGT, FFA, and CFbg in *P. aeruginosa* group increased, and increased with the increase of the *P. aeruginosa* resistance species, while the content of P in *P. aeruginosa* group decreased, and further decreased with the increase of the *P. aeruginosa* resistance species. These results suggest that GGT, P, FFA, and CFbg are closely related to acute appendicitis caused by *P. aeruginosa* infection and may be potential biomarkers. In this study, the children

Table 4. The OR Values of GGT, P, FFA, Cfbg and Their Combination.

Factors	Control Vs. *Pseudomonas aeruginosa*		PAR- Vs. PAR+	
	OR	95% CI	OR	95% CI
GGT	1.1408	1.0386 - 1.2531	1.0468	0.9016 - 1.2154
P	0.0201	0.0025 - 0.1602	0.2001	0.0061 - 6.5142
FFA	5.3870	1.4451 - 20.0809	313.6216	90.1554 - 1778.4525
CFbg	3.4416	1.9576 - 6.0503	1.0174	0.6169 - 1.6781
Conbination	655.9676	109.9204 - 3914.5928	1115.8482	121.2366 - 10270.1414

with negative bacterial culture might also be in the early stage of the disease. Because these children had used antibiotics, and the bacteria had been eliminated in the early stage, resulting in no pathogenic bacteria detected in the culture of appendix exudate or peritoneal pus taken out during the later operation.

With the occurrence of bacterial infection, the inflammatory response in the body is often further triggered (23). By analyzing CRP, an indicator of inflammation, we found that the degree of inflammation in children increased with the infection of *P. aeruginosa* and the increase of drug resistance. GGT is an indicator related to liver injury (24), and the increase in its content in this study may be related to the degree of inflammation caused by infection, or to the liver function damage caused by some substances (endotoxin, etc.) produced by bacteria. As bacteria proliferate in the abdominal cavity, it often affects the intestinal water salt balance and lipid metabolism. Therefore, the increase of FFA in the blood of patients in this study may be caused by the proliferation and release of *P. aeruginosa*, while the decrease of P may be caused by the metabolic consumption of *P. aeruginosa*. Extended spectrum β-lactamases (ESBLs) are produced in most patients with bacterial infection resistant to β-lactamases (25). Therefore, we speculate that the content difference of GGT, P, FFA, and CFbg may be related to the drug-resistant enzyme produced by bacteria, and the enzyme may have a certain impact on the body, but the specific mechanism needs further study.

5.1. Conclusions

This study found that children with acute appendicitis caused by *P. aeruginosa* showed a high resistance rate to antimicrobial agents. Through the combination of GGT, P, FFA, and CFbg, we could effectively determine whether the children with acute appendicitis were infected by P. aeruginosa and resistant to penicillin antibiotics, which could be used as a predictor, and is of great significance for guiding clinicians to choose treatment plans at an early stage.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

Footnotes

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