

Drug resistance analysis of bacterial strains isolated from burn patients

L.F. Wang, J.L. Li, W.H. Ma and J.Y. Li

Inner Mongolia Institute of Burn Research,
The Third Affiliated Hospital of Inner Mongolia Medical University, Baotou,
Inner Mongolia, China

Corresponding author: L.F. Wang
E-mail: lfjycn@yeah.net

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ABSTRACT. This study aimed to analyze the spectrum and drug resistance of bacteria isolated from burn patients to provide a reference for rational clinical use of antibiotics. Up to 1914 bacterial strain specimens isolated from burn patients admitted to hospital between 2001 and 2010 were subjected to resistance monitoring by using the K-B paper disk method. Retrospective analysis was performed on drug resistance analysis of burn patients. The top eight bacterium strains according to detection rate. A total of 1355 strains of Gram-negative (G⁻) bacteria and 559 strains of Gram-positive (G⁺) bacteria were detected. The top eight bacterium strains, according to detection rate, were *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Enterococcus*. Drug resistance rates were higher than 90% in *A. baumannii*, *P. aeruginosa*, *S. epidermidis*, and *S. aureus*, which accounted for 52.2, 21.7, 27.8, and 33.3%, respectively, of the entire sample. Those with drug resistance rates lower than 30% accounted for 4.3, 30.4, 16.7, and 16.7%, respectively. Multidrug-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) accounted for 49.2 and 76.4% of the *S. epidermidis* and *S. aureus*

resistance, respectively. Antibacterial drugs that had drug resistance rates to MRSE and MRSA higher than 90% accounted for 38.9 and 72.2%, respectively, whereas those with lower than 30% drug resistance rates accounted for 11.1 and 16.7%, respectively. The burn patients enrolled in the study were mainly infected with G⁻ bacteria. These results strongly suggest that clinicians should practice rational use of antibiotics based on drug susceptibility test results.

Key words: Burns; Bacteria; Antibiotics; Drug resistance

INTRODUCTION

Anti-infection treatment is an important step during the treatment of burns, and antibiotic application is a crucial means of infection prevention and control. However, along with the widespread use of antibiotics, the bacterium spectrum and bacterial drug resistance have also developed; reinforced multidrug resistance in bacterium results in great difficulties in clinical treatment. Thus, accurate knowledge of the bacterium spectrum, bacterial resistance trend, and changes in drug resistance to antibiotics are essential for determining the rational clinical use of antibiotics (Li et al., 2005a). In this study, the strains and respective drug resistances of bacteria isolated from burn patients admitted to our unit between January 2001 and December 2010 were retrospectively analyzed to provide useful data for determining the rational clinical use of antibiotics.

MATERIAL AND METHODS

Bacterium source

A total of 1914 bacterial strains were isolated from burn patients who were hospitalized between January 2001 and December 2012, including from wound secretions, blood, sputum, and other specimens. Up to 1669 wound secretion specimens (87.2%), 185 sputum specimens (9.7%), 53 blood specimens (2.8%), and 7 drainage fluid specimens (0.4%) were obtained. This study was conducted in accordance with the Declaration of Helsinki.

General methods

According to the “National Guide to Clinical Laboratory Procedures”, the bacteria were identified through analytical profile index system identification, adapted from BioMérieux (France). The results were determined based on the National Committee on Clinical Laboratory Standards (2000 edition). The K-B paper disk agar diffusion method was adopted in the drug sensitivity test. The quality control strains used were standard strains, including *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, and *Pseudomonas aeruginosa* ATCC27853. A portion of the antibacterial drug paper disks was obtained from the Oxoid Company (Basingstoke, Hampshire, England), including imipenem, cefepime, ceftazidime/clavulanic acid, and cefotaxime/clavulanic acid. The remaining drug-sensitive paper disks were purchased from Beijing Tiantan Biological Products Co. Ltd. (Beijing, China).

Diagnostic criteria for drug resistance

The diagnostic criteria for drug resistance were established in accordance with the guidelines set by the National Committee for Clinical Laboratory Standards.

Statistical analysis

Data were analyzed with the WHONET software, version 5.3.

RESULTS

Bacterial separation rate

Up to 1355 strains of gram-negative (G^-) rod bacteria and 559 strains of gram-positive (G^+) rod bacteria were detected, accounting for 70.8 and 29.2% of the sample, respectively. The separation rates of G^- and G^+ rod bacteria in burn patients admitted to hospital between 2001 and 2010 are shown in Figure 1. The distribution of the 1914 bacterial strains is shown in Figure 2.

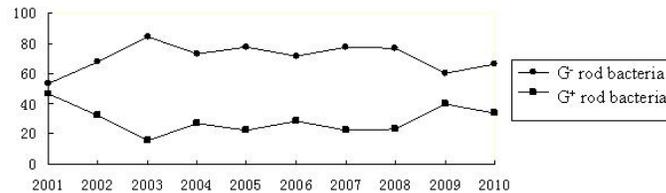


Figure 1. Separation rates of G^- rod bacteria and G^+ rod bacteria (%) during 2001-2010.

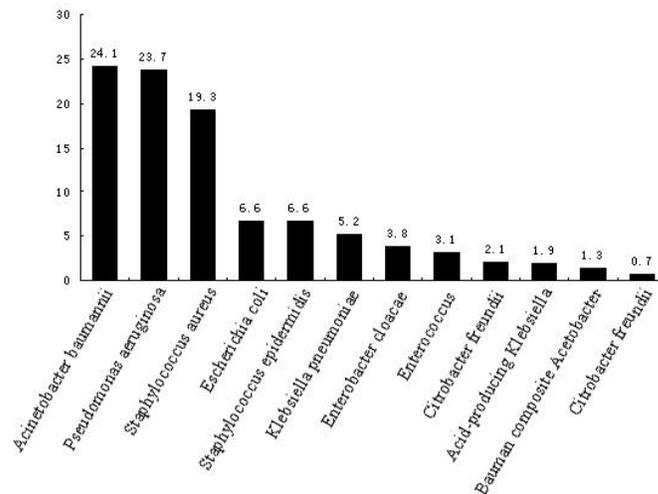


Figure 2. Distribution of 1914 bacterium strains. Note: MRSA was accounted for 76.4% in *Staphylococcus aureus* and MRSE was accounted for 49.2% in *Staphylococcus epidermidis*.

Bacterial detection rate

According to the detection rate, the top eight bacteria were *Acinetobacter baumannii*, *P. aeruginosa*, *S. aureus*, *E. coli*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Enterococcus*. The distribution of bacteria throughout the period of 2001 to 2010 is shown in Table 1.

Table 1. Distribution of the top eight bacterial strains according to detection rate in burn patients during 2001-2010 (detection number/detection rate %).

Years	Total	aba	pae	sau	eco	sep	kpn	ecl	ent
2001	32	0/0	6/18.8	1/3.1	4/12.5	10/31.3	1/3.1	5/15.6	4/12.5
2002	78	8/10.3	17/21.8	15/19.2	12/15.4	6/8.0	4/5.1	7/9.0	2/2.6
2003	94	14/14.9	31/33.0	8/8.5	5/5.3	3/3.2	17/18.1	10/10.6	2/2.1
2004	81	22/27.2	17/21.0	5/6.2	7/8.6	15/18.5	8/9.9	3/3.7	2/2.5
2005	127	49/38.6	20/15.7	15/11.8	9/7.1	10/7.9	3/2.4	10/7.9	2/1.6
2006	240	56/23.3	58/24.2	21/8.8	29/12.1	35/14.6	12/5.0	20/8.3	13/5.4
2007	292	66/22.6	58/19.9	31/10.6	17/5.8	30/10.3	16/5.5	2/0.7	4/1.4
2008	317	64/20.2	147/46.4	68/21.5	5/1.6	0/0	3/0.9	5/1.6	6/1.9
2009	408	99/24.3	77/18.9	150/36.8	18/4.4	11/2.7	27/6.6	10/2.5	2/0.5
2010	245	84/34.3	22/9.0	55/22.4	21/8.6	6/2.4	17/6.9	1/0.4	22/9.0
Total	1914	462/24.1	454/23.7	369/19.3	127/6.6	126/6.6	100/5.6	73/3.8	59/3.1

Drug resistance of G⁻ rod bacteria in the top eight bacterial strains according to detection rate

The drug resistances of G⁻ rod bacteria in the top eight bacteria according to detection rate are shown in Table 2. The antibacterial drugs with a drug resistance rate to *A. baumannii* lower than 30% only included cefoperazone/sulbactam, accounting for 4.3%; those with drug resistance rates higher than 90% included ampicillin, aztreonam, SMZCo, chloramphenicol, piperacillin, gentamycin, cefoperazone, ceftriaxone, ceftazidime, ceftazolin, tobramycin, and cefotaxime, accounting for 52.2%. The antibacterial drugs with drug resistance rates to *P. aeruginosa* lower than 30% included amikacin, aztreonam, piperacillin/tazobactam, cefepime, cefoperazone/sulbactam, ceftazidime, and imipenem, accounting for 30.4%; those with higher than 90% drug resistance rates included ampicillin, ampicillin/sulbactam, SMZCo, chloramphenicol, and ceftazolin, accounting for 21.7%.

Drug resistance of G⁺ rod bacteria

The drug resistances of G⁺ rod bacteria in the top eight bacteria according to detection rate are shown in Table 3. The antibacterial drugs with lower than 30% drug resistance rate to *S. aureus* and *S. epidermidis* accounted for 16.7%; those with higher than a 90% drug resistance rate accounted for 33.3% and 27.8%, respectively. The antibacterial drugs with a lower than 30% drug resistance rate to multidrug-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) accounted for 16.7 and 11.1%, respectively, and those with higher than 90% drug resistance rate accounted for 72.2 and 38.9%, respectively.

Table 2. Drug resistance of G⁻ rod bacteria in the first eight bacterial strains according to detection rate (Drug resistance rate %/Detection number).

Antibiotics	G ⁻	aba	pac	eco	kpn	ecl
Amikacin	46.6 /1119	82.5/354	24.6/414	19.4/108	25.3/99	58.8/68
Amoxicillin/clavulanic acid	81.8/11	-	75.0/4	100/1	0/1	100/3
Ampicillin	96.3/408	91.5/59	93.9/33	98.2/112	97.9/96	100/68
Ampicillin/Shubatan	82.5/63	42.9/14	100/37	33.3/3	100/3	75.0/4
Aztreonam	54.2/1123	90.8/358	22.9 /415	57.8/109	39.4/99	63.2/68
Cotrimoxazole	90.0/448	90.4/146	93.8/210	92.9/28	52.4/21	100/11
Ciprofloxacin	60.5/1106	83.6/415	38.9/350	76.9/108	22.4/98	53.3/60
Chloramphenicol	87.5/48	100/18	95.8/71	50.0/2	0/1	-
Piperacillin	72.2/1170	91.8/416	53.4/410	74.1/112	50.5/97	87.9/66
Piperacillin/tazobactam	19.3/765	36.4/316	10.8/287	1.8/56	0/57	3.6/28
Gentamicin	80.8/1144	91.3/413	77.8/383	86.5/111	50.0/94	83.6/67
Cefepime	49.1/1143	85.9/417	16.4/408	59.6/99	25.0/88	32.3/65
Cefuroxime sodium	71.6/285	88.9/36	84.0/25	70.3/74	56.0/84	80.6/36
Cefoperazone	71.2/1040	95.4/410	56.4/388	37.7/69	47.6/82	61.4/44
Cefoperazone/Shubatan	6.4/469	5.0/200	3.8/130	5.0/40	0/45	0/36
Ceftriaxone	71.0/572	95.4/218	58.3/84	62.5/80	45.2/84	63.4/41
Ceftazidime	56.8/1180	93.3/418	21.8/404	57.1/112	37.4/99	66.2/71
Cefazolin	80.7/394	96.2/52	95.7/23	74.1/108	58.4/89	98.5/68
Tobramycin	80.8/532	94.0/183	75.2/318	100/8	0/9	100/1
Imipenem	32.2/1142	57.0/426	22.6/424	0/112	2.0/98	1.5/68
Ofloxacin	53.5/86	80.0/5	44.1/68	100/5	100/3	80.0/5
Levofloxacin	40.6/668	39.6/260	60.8/186	62.2/45	8.6/58	20.0/20
Cefotaxime	65.1/728	90.8/217	53.3/152	58.9/112	37.8/98	70.4/71

Table 3. Drug resistance of G⁺ rod bacteria in the first eight bacterial strains according to detection rate (Drug resistance rate %/Detection number).

Antibiotics	G ⁺	Sau	MRSA	Sep	MRSE	Ent
Amikacin	88.0/75	83.3/30	92.0/3	90.9/44	100/7	100/1
Azithromycin	95.2/273	95/220	99.4/168	96.2/53	96.3/27	-
Ampicillin	67.8/90	96.6/29	100/20	94.1/17	100/12	41.5/41
Oxacillin	76.6/320	79.5/259	99.5/206	63.3/60	100/38	100/1
Cotrimoxazole	77.9/367	79.2/268	88.4/207	73.7/95	73.5/68	100/4
Erythromycin	93.9/488	94.4/354	98.6/277	96.7/92	96.2/53	84.6/39
Ciprofloxacin	79.4/383	82.6/298	96.3/240	65.7/70	70.0/40	80.0/15
Clindamycin	83.7/454	90.4/354	97.5/276	59.1/93	71.7/53	100/4
Rifampicin	36.2/469	35.2/341	42.5/268	27.8/97	48.2/56	76.6/30
Chloramphenicol	14.5/399	9.0/312	8.0/264	35.0/60	48.6/37	37.5/24
Norfloxacin	71.4/7	100/2	100/2	75.0/4	75.0/4	0/1
Gentamicin	78.5/362	84.6/279	96.8/219	58.7/75	57.4/47	50.0/8
Teicoplanin	1.7/239	0/200	0/167	0/17	0/2	19.0/21
Cefazolin	83.3/144	91.1/112	97.1/105	54.8/31	100/10	100/1
Vancomycin	0.8/520	0/361	0/283	0/102	0/61	7.4/54
Ofloxacin	82.6/23	81.8/11	90.0/10	66.7/6	66.7/6	100/3
Levofloxacin	73.9/207	79.6/162	91.8/134	56.8/37	42.1/19	50.0/6
Penicillin G	80.4/582	81.5/427	99.6/279	96.0/101	100/59	43.1/51

DISCUSSION

Retrospective analysis was performed to identify the bacteria and their drug resistances isolated from burn patients in our burn department. The results showed that the bacteria spectrum has changed in the past 10 years, and bacteria show different degrees of resistance to antibiotics. From 2001 to 2010, 1914 bacterial strains were detected from burn patients, including from wound secretions, blood, sputum, and other specimens. The top eight bacteria were identified

as *A. baumannii* (462 strains), *P. aeruginosa* (454 strains), *S. aureus* (369 strains, including 282 MRSA strains), *E. coli* (127 strains), *S. epidermidis* (126 strains, including 62 MRSE strains), *K. pneumoniae* (100 strains), *E. cloacae* (73 strains), and *Enterococcus* (59 strains). Twice as many G⁻ rod bacteria (70.8%) were detected as G⁺ rod bacteria (29.2%), which is a higher rate than any survey data reported prior to 2000 (Xu et al., 2002), but is similar to those reported after 2000 (Wei and Liu, 2006). These findings indicate that G⁻ rod bacteria remain the dominant bacteria in burn infections in XX hospital. In the past three years, *P. aeruginosa*, *S. aureus*, and *A. baumannii* alternately occupied the top three places, which is similar to rankings previously cited both in China and elsewhere (Wei and Liu, 2006; Essayagh et al., 2011). Thus, the clinical treatment of burn infections should receive more attention.

Along with the widespread use of the three generations of cephalosporin, ceftazidime, and carbapenems, imipenem, the drug resistance rate units used in these drugs has noticeably increased; the more intractably conditioned pathogens are typically screened out under the pressure of antibiotics, which is almost the same for patients in burn wards and intensive care units (Xiao, 2004). Although new antibiotics are constantly being developed, bacterial drug resistance is strengthening, which causes difficulties in clinical treatment of burn infections. In this study, G⁻ rod bacteria accounted for five of the top eight detected bacterial strains. *A. baumannii* was the most frequently detected, followed by *P. aeruginosa*. Among the detected G⁻ rod bacteria from burn patients, *A. baumannii* exhibited an annually increasing trend that showed a higher detection rate compared with previous reports in literature (Xu et al., 2001, 2002; Essayagh et al., 2011; Alp et al., 2012). *A. baumannii* is a G⁻ rod bacterium that is not fermented by carbohydrates, and is widely distributed in nature, hospital environments, and human skin. *A. baumannii* is a conditioned pathogen, 7% of which is located in human pharyngeal organs (Li et al., 2005b). With the wide application of antibacterial drugs, bacterial drug resistance is sharply increasing and multi-drug resistant bacterial strains are evolving, causing great difficulties in clinical treatment. Hospital infections caused by *A. baumannii* are generally on the rise, and this is the main pathogen reported in some intensive care units (Pimentel et al., 2005). The infection rate of multi-drug resistant *A. baumannii* has drawn clinical attention (Oliveira and de Lencastre, 2002). Therefore, detailed knowledge of its distribution characteristics and dynamic changes in its drug resistance spectrum is important.

In this study, isolated *A. baumannii* showed a relatively low drug resistance rate to cefoperazone/sulbactam (5%), but higher drug resistance rates to the other commonly used antibiotics, such as imipenem, which was 57% at XX hospital compared with less than 10% reported previously (Cheng et al., 2003). β -lactam antibiotics were highly resistant, whereby almost all of them exhibited 90% drug resistance. The drug resistance mechanism of *A. baumannii* to β -lactam antibiotics mainly involves the production of β -lactamase. Acinetobacter could easily produce drug resistance by combining plasmids. A variety of resistant plasmids coexist, such as plasmid-mediated TEM-1 and TEM-2, TEM-2 β -lactamase, and chromosome-mediated β -lactamase; the change in penicillin-binding proteins and the permeability decrease of outer membrane proteins can also result in drug resistance (Yang and Li, 2004; Yu and Li, 2006; Zhang and Chen, 2007). The data of the National Gram-Negative Resistance Survey indicated that from 1994 to 2001, *P. aeruginosa* ranked first among the infected G⁻ bacteria in all hospitals; moreover, the sensitivities of the antibiotics declined (Wang and Chen, 2003).

In this study, the G⁻ rod bacteria had lower drug resistance to cefoperazone/sulbactam and piperacillin/tazobactam mainly because the antibiotics belonged to the restricted-use varieties in

the unit, and were only used to treat severe infections with indications of bacterial drug sensitivity.

In this study, the antibacterial drugs with a higher than 90% drug resistance rate of the detected *P. aeruginosa* accounted for 21.7%, which is lower than results previously reported (Wei and Liu, 2006). Drugs with lower than 30% resistance rates were only amikacin, aztreonam, piperacillin/tazobactam, cefepime, cefoperazone/sulbactam, ceftazidime, and imipenem, with drug resistance rates of 24.6, 22.9, 10.8, 16.4, 3.8, 21.8, and 22.6%, respectively. The relatively low resistance level observed was related to the long-term and persistent use of the results of bacterial culture drug sensitivity tests in our burn ward to guide the clinical use of antibiotics and the strict antibiotic use system. None of the drugs listed above were clinical first-line medicines used in our department. With multi-drug resistance, *P. aeruginosa* could naturally resist multiple antibiotics and easily develop drug resistance in the course of antibiotic treatment. A variety of drug resistance mechanisms are known, including generation of β -lactamase, permeability decrease of the bacterial outer membrane, and changes in the structure and function of bacterial proteins (Hirakata and Izumikawa, 1998; Xu et al., 2001; Sun et al., 2009; Gu et al., 2005). Therefore, *P. aeruginosa* infections should be treated with reasonable use of antibiotics based on the epidemiological characteristics and bacterial culture results in this unit to avoid the generation of drug-resistant strains.

In this study, the detection rate of *S. aureus* in G⁺ rod bacteria ranked first, accounting for 66.0%. *S. aureus* is usually present in human skin or nasopharyngeal organs. *S. aureus* is the most common pathogen in festering infections that could cause all kinds of infections; it is particularly common in elderly and critical patients. MRSA infections are mainly associated with pneumonia, skin, or soft tissue infections, blood infections, and bone infections. MRSA is spread by direct or indirect contact with afflicted patients. MRSA was first identified in Britain in 1961. The clinical detection rate of MRSA annually increased in a span of 50 years. The extensive application of broad-spectrum antibiotics and the abuse of antibiotics have made MRSA an important pathogen in hospital infections. The harm caused by MRSA infections has drawn increasing clinical attention. In addition, the drug resistance of MRSE should not be ignored. This study showed that antibacterial drugs with a higher than 90% drug resistance rate to MRSA and MRSE accounted for 72.2 and 38.9%, respectively; those with drug resistance rates to MRSA lower than 30% included chloramphenicol, teicoplanin, and vancomycin, whereas those with drug resistance rates to MRSE lower than 30% included only teicoplanin and vancomycin. In this study, *Enterococcus* was found to be resistant to the drugs teicoplanin and vancomycin, but with lower drug resistance rates, which may be related to the VanC type gene (Qian and Ning, 2011).

In view of the increase in bacterial drug resistance, attention should be given to the structural changes of pathogenic bacteria in the environment. Clinicians should focus closer attention to the local flora changes in this unit and consider future developmental trends to improve the activity and predictability of clinical anti-infection treatment. Bacterial culture susceptibility indicators are still important bases for the selection of antibiotics, and therefore, in the anti-infection treatment of burns. The specifications of antibiotics should be strictly implemented to use drugs appropriately.

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Conflicts of interest

The authors declare no conflict of interest.

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