

## ***In vitro* Susceptibility Testing to 14 Antimicrobial Agents of Bacterial Strains Isolated from Infected Wounds**

**Mahmud J. Abussaud and Mamdoh M. Megdam**

*Department of Biological Sciences,  
Yarmouk University, Irbid, Jordan*

**ABSTRACT.** *In vitro* susceptibility testing of bacteria isolated from infected wounds from different sources in Basma Hospital, Irbid, demonstrated that all gram-negative bacteria were resistant to novobiocin and sensitive to gentamycin. *Staphylococcus aureus* strains isolated from infected wounds were mostly either resistant to penicillin G and ampicillin or sensitive to gentamycin. Variable susceptibilities were observed for the tested antibiotics. As the differences between the resistant pattern of *S. aureus*, isolated from different sources, was statistically significant, no single source of *S. aureus* can be considered to be responsible for the spread of infection. Therefore, the bacteria must have been originated from multiple sources.

It is very difficult to make surgical procedures completely aseptic. The sources of wound contamination are, in most cases, not clear (Linton 1961). Generally, micro-organisms responsible for post operative wound infections come either from endogenous sources, *i.e.* skin, urinary and gastro-intestinal tracts, or from exogenous sources, *i.e.* the environment or the operating team (Black 1966, Dyas *et al.* 1982, Simmons 1983, and Kundsinn 1980).

Contamination of wound could be minimized by cleaning the skin with soap, water 70% alcohol and ether the day before the operation and again just before the operation (Lovell 1946). Iodophores and hexachlorophore are also effective for pre-operative cleaning of skin (Simmons 1983). In the early days of antibiotics, it was hoped that their use as a prophylactic would eliminate the chances of sepsis formation. However, this was not the case and instead it was shown that the use of various antibiotics potentiated the growth of resistant organisms.

It is generally agreed each hospital and each surgical ward has its own conditions which may contribute towards increasing or decreasing the post

operative infection rate.

In a study at Princess Basma hospital in Irbid, Jordan to determine the rate of post operative wound sepsis (unpublished work), a total of 235 *Staphylococcus aureus* strains were isolated from different sources.

The aim of this study was to determine the *in vitro* susceptibility pattern of these organisms to different antimicrobial agents.

## Material and Methods

### Antibiotic Disks

All of the following antibiotic disks were obtained from Biomerieux, Germany: Ampicillin (10 µg), Carbencillin (100 µg), Cephaloridine (30 µg), Chloramphenicol (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Kanamycin (30 µg), Nalidixic acid (30 µg), Nitrofurantion (300 µg), Novobiocin (5 µg), Penicillin G (10 Iµ), [Polymyxin B (300 Iµ)], Streptomycin (10 µg) and Tetracycline (10 µg).

### Reference Strains

The following American Type Culture Collection Strains (ATCC) were used: *S. aureus* (# 25922), *Pseudomonas aeruginosa* (# 27853) and *Escherichia coli* (# 25922).

### Antibiotic Susceptibility Testing by Standardized Single Disk Methods

The technique recommended by the National Committee for Clinical Laboratory Standard (NCCLS 1976) was followed.

## Results

The resistance pattern of gram-negative bacteria isolated from infected wounds to antimicrobial agents is shown in Table 1. The three isolates of *E. coli* were fully resistant to Novobiocin and showed 66.7% resistance to Ampicillin, Carbencillin and Tetracycline. *Proteus vulgaris* was fully resistant to most of the antibiotics, used in this study, except Gentamycin and Nalidixic acid. *Pseudomonas aeruginosa* showed full resistance to most of the antibiotics except Carbenicillin, Gentamycin, Kanamycin and Polymyxin B. *Enterobacter aerogenes* was completely resistant to Ampicillin, Nitrofurantion, Novobiocin and Tetracycline. *Aeromonas hydrophillia* showed full resistance to Ampicillin and Novobiocin, while no resistance was found to the rest of the antibiotics studied.

**Table 1.** Resistance patterns of gram-negative bacteria isolated from infected wounds.

Bacterium	Ampicillin	Carbenicillin	Chloramphenicol	Gentamycin	% Resistance						
					Kanamycin	Nalidixic acid	Nitrofurantion	Novobiocin	Polymyxin B	Streptomycin	Tetracycline
<i>Escherichia coli</i>	66	66	33	0.0	33.3	0.0	0.0	100.0	0.0	33.3	66.7
<i>Proteus vulgaris</i>	100	66	100	0.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0
<i>Pseudomonas aeruginosa</i>	100	0	100	0.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0
<i>Enterobacter aerogenes</i>	100	0	0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	100.0
<i>Aeromonas hydrophilia</i>	100	0	0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0

Table 2 shows the susceptibility pattern of *S. aureus* isolated from different sources to Ampicillin, Cephaloridine, Chloramphenicol, Erythromycin, Gentamycin, Kanamycin, Penicillin G, Streptomycin and Tetracycline. *S. aureus* isolated from the nose and skin of infected patients, from the air and from articles from the intensive care unit and dressing room, showed the highest percentage resistance to Ampicillin (100%), followed by strains isolated from the noses of patients (93.4%), and from surgical wounds (72.7%). The majority of *S. aureus* isolated from all sources were sensitive to Cephaloridine. The highest resistant strains were among those isolated from the intensive care unit (25%), followed by those from surgical wounds (18.2%).

*S. aureus* isolated from surgical wounds showed the highest resistance to chloramphenicol (36.6%), followed by those isolated from the intensive care unit (25%). Most *S. aureus* isolated from other sources were sensitive to Chloramphenicol.

*S. aureus* isolated from the intensive care unit and the dressing room showed the highest degree of resistance to Erythromycin (50%), followed by strains isolated from the operating theatre and surgical wounds (42.9% and 36.6% respectively). While the majority of *S. aureus* isolated from all sources were sensitive to Gentamicin and Kanamycin [except those from surgical wounds which were resistant to Kanamycin (36.4%)] they were all resistant to penicillin.

*S. aureus* isolated from surgical wounds and from the operating theatre showed the highest percentage resistance to Streptomycin (36.6 and 35.7%

respectively) while those isolated from other sources were sensitive. The highest percentage resistance to Tetracycline was found in strains isolated from skin of infected patients (66.7%), the operating theatre and from the dressing room (50% each), while the rest of the isolated strains showed a high percentage of sensitivity to this antibiotic.

**Table 2.** Resistance patterns of *S. aureus* strains isolated from infected wounds and different sources.

Sources of isolates	No. of isolates	Ampicillin	Cephaloridine	% of Resistance						
				Chloramphenicol	Erythromycin	Gentamycin	Kanamycin	Penicillin G.	Streptomycin	Tetracycline
Surgical wounds	11	72.7#	18.2#	36.6#	36.6#	9.1#	36.6#	81.8#	36.6#	27.3#
Noses of patients	61	93.4	3.3	5.0	8.2	4.9	4.9#	95.1	4.9	18.0#
Skin of patients	27	66.7#	0.0	7.4	22.2	0.0	0.0	74.1#	18.0	37.0#
Noses of infected patients	5	100.0	0.0	0.0	0.0	20.0	20.0	100.0	18.0	20.0#
Skin of infected patients	3	100.0	0.0	7.4	0.0	0.0	0.0	100.0	0.0	66.7
Noses of hospital staff	6	100.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	16.7#
Hands of hospital staff	5	40.0	0.0	0.0	20.0	0.0	0.0	60.0	20.0	20.0#
Operating theatre	98	50.0	0.0	7.1	42.9#	0.0	14.0	64.0	35.7#	50.0#
Intensive care unit	15	100.0	25.0#	25.0#	50.0#	0.0	25.0#	100.0	25.0#	25.0#
Dressing room	15	100.0	0.0	0.0	50.0#	0.0	0.0	100.0	0.0	50.0#

All pairwise comparisons between data from surgical wounds and the rest of the sources of the isolates were significant at 5% level of probability using X<sup>2</sup>-test, except those indicated by (#).

The susceptibility pattern of the bacteria isolated from infected wounds as the minor organism (Table 3) was also studied, and was as follows: *Proteus inconstans* was fully resistant to Ampicillin, Cephaloridine, Novobiocin and Polymyxin B, and fully sensitive to the rest of the antibiotics; *Bacillus cereus* was resistant to Ampicillin, Novobiocin, Penicillin G./Polymyxin B, and Streptomycin, while fully sensitive to Cephaloridine, Gentamicin, Erythromycin and Tetracycline; *Bacillus licheniformis* was fully sensitive to most of the antibiotics except to Novobiocin and Penicillin G. *Alkaligenes faecalis* was fully sensitive to most of the antibiotics except to Cephaloridine, Chloramphenicol, and Novobiocin.

*S. epidermidis* was fully sensitive to Gentamycin, Kanamycin and Streptomycin and fully resistant to the others. *Flavobacterium* showed full sensitivity to most of the antibiotics except to Cephaloridine and Novobiocin.

A comparison was made between the percentage of resistant strains from surgical wounds and from each of the other sources.

The highest percentage of resistant strains isolated from surgical wounds and other sources was to Penicillin G, followed by Ampicillin, while the lowest was to Gentamicin followed by Cephaloridine (Table 2). *S. aureus* showed variable resistance to the rest of the antibiotics.

**Table 3.** Total major and minor organisms isolated from infected wounds out of 504 operations.

Bacterium	Pure Major	Mixed		Total
		Major	Minor	
<i>Staphylococcus aureus</i>	4	6	2	12
<i>Escherichia coli</i>	2	1	0	3
<i>Proteus vulgaris</i>	1	1	1	3
<i>Pseudomonas aeruginosa</i>	0	1	0	1
<i>Enterobacter aerogenes</i>	1	0	0	1
<i>Aeromonas hydrophilia</i>	0	1	0	1
<i>Staphylococcus epidermidis</i>	0	1	2	2
<i>Streptococcus faecalis</i>	0	0	3	3
<i>Bacillus cerus</i>	0	0	1	1
<i>Bacillus licheniformis</i>	0	0	1	1
<i>Proteus inconstans</i>	0	0	1	1
<i>Alkaligenes faecalis</i>	0	0	1	1
<i>Aerococcus viridans</i>	0	0	1	1
<i>Flavobacterium</i> group 1	0	0	2	2
Total	8	10	15	33

### Discussion

The highest percentage of resistance of *S. aureus* strains isolated from various sources were to Penicillin G and Ampicillin. The lowest percentage of resistance was to Gentamycin and Cephaloridine. Resistance to both antibiotics may be mainly due to the elaboration of penicillinase, which inactivates them. The susceptibility pattern of the major organisms isolated from infected wounds, other than *S. aureus*, to various types of antibiotics showed multiresistant strains. This pattern suggested that R-Plasmids or other mechanisms, *i.e.* the alteration of the target site, where the antibiotic is usually bound, and the development of impermeability of the bacterial cell wall to antibiotics could be responsible for this multiresistance (Hau *et al.* 1983).

Two hundred and thirty five cultures of *S. aureus* were studied to find out the sources of bacteria responsible for post-operative wound sepsis. This was done by comparing the resistance of bacteria isolated from surgical wounds and those isolated from patients, surgical staff members and from the environment in the operating theatre, the intensive care unit and the dressing room. Hasselgren and Holm (1981) studied 112 patients to find the sources of pacteria causing wound infection. They found that the patients themselves were the sources of the bacteria in all cases of wound infection. Howe (1956) reported that the increase in post-operative sepsis was due to high penicillin resistant carrier rate in hospital personnel and patients as a result of widespread use of penicillin. Burke (1963) reported the scrub team and patients were the major source of wound contamination. Wemambu (1981) compared the resistance of *S. aureus* isolated from the nose of surgeons and the operating unit personnel to different types of antibiotics. He found that the staphylococcal infection was probably acquired from the surgical staff members, although acquisition from other infected patients was not impossible.

In this study, we were unable to do phage typing to better pin point the sources of *S. aureus* because of the lack of phages. However, statistical analysis showed a significant difference ( $P < 0.05$ ) between resistance of *S. aureus* strains isolated from the infected wounds and those isolated from the patients, hospital staff members, operating theatre, intensive care unit and the dressing room. It is, therefore, possible that *S. aureus* strains isolated from infected wounds might have originated from multiple sources. Similar results were reported by Dyas *et al.* (1982).

This study indicated that Gentamycin was the most effective antimicrobial agent against gram-negative bacteria and *S. aureus* strains isolated from infected wounds during the study (Tables 1 and 2). It also showed the importance of antimicrobial susceptibilty testing of the bacterial isolates in order to help in the choice of the appropriate antibacterial therapy for the treatment of wound infection and to determine the trends in the development of bacterial antibiotic resistance.

### References

- Black, T. (1966) The bacterial flora of the skin and its relation to post-operative wound infection, *Trans. Soc. Occ. Med.* **16**: 18-23.
- Burke, J.F. (1963) Identification of the sources of staphylococcal contaminating the surgical wound during operation, *Amer. Surg.* **158**: 898-904.
- Dyas, A.C., Eden, P.J., Eastwood, D., Bentley, S., Burdon, D.W., Alexander-Williams, J. and Keighley, M.R.B. (1982) Sources of staphylococcal wound sepsis in surgical patients, *J. Hosp. Infect.* **3**: 345-350.

- Hasselgren, P.O. and Holm, J.** (1981) Sources and routes in post-operative wound infections, *Acta Chir. Scand.* **197**: 99-103.
- Hau, T., Nishikawa, R. and Danziger, L.H.** (1983) Antibiotics in surgery, *Surg. Ann.* **15**: 177-205.
- How, C.W.** (1956) Prevention and control of post-operative wound infections owing to *Staphylococcus aureus*, *New Eng. J. Med.* **255**: 787-794.
- Kundsinn, R.B.** (1980) Documentation of airborne infection during surgery, *Ann. N.Y. Acad. Sci.* **353**: 255-261.
- Linton, R.R.** (1961) The prophylactic use of antibiotics in clean surgery, *Surg. Gynecol. Obstet.* **112**: 218-220.
- Lovell, D.L.** (1946) Pre-operative skin preparation with reference to surface bacterial contaminants and resident flora, *Surg. Clin. N. Amer.* **26**: 1053-1059.
- NCCLS Subcommittee on Antimicrobial Susceptibility Testing Performance Standards for antimicrobial disk susceptibility tests** (1976) The National Committee for Clinical Laboratory Standards, Approved standard.
- Simmons, B.P.** (1983) CDC guidelines for the prevention and control of nosocomial infections and surgical wound infections, *Amer. J. Infect. Control* **11**: 133-143.
- Wemambu, S.N.C.** (1981) Wound infection and nasal colonization of staff with *Staphylococcus aureus* in Benin, *Nigeria. J. Hosp. Infect.* **2**: 259-260.

(Received 09/02/1986;  
in revised form 29/06/1986)

## حساسية الجراثيم المعزولة من الجروح الملوثة لأربعة عشر نوعاً من المضادات الحيوية

محمود جدوع أبو السعود و ممدوح مفلح مقدم

دائرة علوم الحياة - جامعة اليرموك - أربد - الأردن

تم اختبار حساسية ٢٤٦ عزلة من بكتيريا المكورات العنقودية *St. aureus* التي عزلت من جروح ملوثة ومن مصادر أخرى متنوعة، لعدد من المضادات الحيوية المختلفة، وتبين أن معظمها مقاوم للبنسلين والأمبسلين وحساساً للجنتميسين، أما بالنسبة لبقية المضادات الحيوية فقد كانت حساسية الجراثيم لها متباينة. كذلك تم فحص حساسية عدد من الجراثيم السالبة المعزولة من الجروح الملوثة، وتبين أن معظمها مقاوم للنوفويوسين وحساساً للجنتميسين. اتضح من هذه الدراسة أنه لا يوجد مصدر واحد من جميع المصادر التي درست يمكن أن يتهم بأنه المسؤول عن تلويث العمليات الجراحية، بل أن كل هذه المصادر كانت مشتركة في المسؤولية عن عمليات التلويث هذه.