

Methods of Susceptibility Testing in Indonesia

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Abstrak

Indonesia merupakan negara maritim yang terdiri atas ribuan pulau. Pulau-pulau ini dihuni oleh lebih kurang seratus delapan puluh juta penduduk yang terkonsentrasi di beberapa pulau besar, antara lain pulau Jawa, Sumatra, Bali, Sulawesi dan Kalimantan. Karena kasus infeksi masih sangat banyak dijumpai di Indonesia, maka dapat dimengerti mengapa hampir semua jenis antibiotik dipakai di sini. Sebagai akibatnya beberapa bulan atau beberapa tahun kemudian mulai muncul sejumlah strain kuman yang tahan atau resisten terhadap obat tersebut. Dengan keadaan yang demikian akan terasa betapa pentingnya suatu laporan pola kepekaan kuman yang dibuat secara akurat dan diumumkan secara teratur. Pada saat ini ada kira-kira delapan laboratorium pada universitas negeri di Indonesia yang secara aktif mengerjakan tes kepekaan kuman. Kebanyakan laboratorium tersebut menggunakan cara difusi cakram Kirby-Bauer yang dianjurkan oleh National Committee for Clinical Laboratory Standards (NCCLS) dari Amerika Serikat. Bagian Mikrobiologi FKUI berharap bahwa cara ini dapat dipakai sebagai cara baku, sehingga hasil tes kepekaan kuman dari berbagai tempat di Indonesia dapat dibandingkan. Konsentrasi hambat minimum suatu antibiotik ditentukan dengan menggunakan cara dilusi lempeng atau tabung, namun pemeriksaan ini tidak dikerjakan secara rutin dalam pelayanan pasien sehari-hari, hanya untuk penelitian atau berdasarkan permintaan khusus. Sedangkan produksi enzim β -laktamase dideteksi dengan cara cakram atau yodometri.

Abstract

Indonesia is a maritime country consisting of thousands of islands. These islands are inhabited by about 180 million people concentrated in several big islands, i.e. Java, Sumatra, Bali, Sulawesi, and Kalimantan. Since cases of infection are very numerous, understandably almost all kinds of antibiotics are being used. Consequently months or years later resistant strains emerged. It seems that regular and accurate reports of susceptibility patterns of antibiotics are urgent. There are at present about eight state university laboratories actively performing antimicrobial sensitivity testing. Most laboratories use the Kirby-Bauer agar disk diffusion method recommended by the NCCLS (National Committee for Clinical Laboratory Standards). We expect this will be the standard method test in Indonesia, so the results are comparable for all regions of the country. Although not routinely performed, the minimum inhibitory concentration is measured by using the plate dilution or tube dilution method. While β -lactamase production is detected by using the disk or iodometric method.

Keywords : Susceptibility testing, Infectious diseases, Resistant strains, Antibiotics

INTRODUCTION

Infectious diseases are still a major problem in Indonesia. The frequent and irrational use of antibiotics might cause the emergence of resistant strains. Many groups of antibiotics are available in this country, i.e. the sulfonamides, tetracyclins, β -lactam aminoglycosides, macrolides, quinolones, etc. General practitioners are free to choose one of those drugs for the treatment of their patients.

Today many laboratories try to anticipate the problem of drug resistance by preparing themselves to perform the sensitivity testing. For academic reasons the university laboratories especially the department of

microbiology of the medical schools had been looking into these problems earlier. Unfortunately antibiotic monitoring and reporting has not been done regularly, but with the recent awareness of nosocomial infection problems, its role are becoming more prominent. On the other hand more attention have to be paid for the standardization of antibiotic sensitivity testing, since the same language is needed to interpret whether an isolate is sensitive or resistant to a certain antibiotic.

PRESENT CONDITION

Indonesia with about 180 million people has twenty seven provinces. Not all of health laboratories in each

province are familiar with sensitivity testing, even though the government tried to improve the condition. In collaboration with the government (The Department of Health, Republic of Indonesia), the Department of Microbiology, Faculty of Medicine, University of Indonesia has organized a technical training course, including sensitivity testing, attended by laboratory technicians from twenty seven provinces in August 1992 (Table 1). It is expected that all technicians will be familiar with the Kirby-Bauer agar disk diffusion methods in measuring the in vitro susceptibility of bacterial isolates to antimicrobial agents, and we hope that this standard method will be used routinely for sensitivity testing. Fortunately this table showed that 81.5% (twenty two out of twenty seven provinces) of the government health laboratories used the Kirby-Bauer agar disk diffusion method, although totally they only performed 97 sensitivity testings per day.

Table 1. Sensitivity testing performed in The Health Laboratories of The Department of Health in Indonesia 1992

No.	Name of the province	Testing method	Specimens per day
1.	Daerah Istimewa Aceh	nm	nm
2.	Sumatra Utara	KB	23
3.	Sumatra Barat	nm	14
4.	Jambi	KB	nm
5.	Sumatra Selatan	KB	12
6.	Bengkulu	KB	nm
7.	Riau	KB	10
8.	Bandar Lampung	KB	12
9.	Daerah Khusus Ibukota	nm	nm
10.	Daerah Istimewa Yogyakarta	KB	43
11.	Jawa Barat	KB	12
12.	Jawa Tengah	KB	nm
13.	Jawa Timur	KB	7
14.	Bali	KB	nm
15.	Kalimantan Barat	KB	12
16.	Kalimantan Tengah	KB	nm
17.	Kalimantan Selatan	KB	nm
18.	Kalimantan Timur	KB	nm
19.	Nusa Tenggara Barat	KB	19
20.	Nusa Tenggara Timur	KB	11
21.	Sulawesi Selatan	KB	21
22.	Sulawesi Tenggara	md	nm
23.	Sulawesi Tengah	KB	1
24.	Sulawesi Utara	KB	nm
25.	Maluku	KB	nm
26.	Irian Jaya	md	nm
27.	Timor Timur	KB	nm
Total number of specimens per day			97

Data collected during The Technical Training on Quality Control of Preparing Media, Staining, Reagents, and Sensitivity Testing in Jakarta on August 19 - September 1, 1992. (KB = Kirby-Bauer; nm = not mentioned; md = modification).

From all over the country there are 8 state university laboratories performing sensitivity testing actively. They are the departments of microbiology of the universities, namely University of North Sumatra in Medan, University of Indonesia in Jakarta, University of Padjadjaran in Bandung, University of Gajah Mada in Yogyakarta, University of Diponegoro in Semarang, University of Airlangga in Surabaya, University of Udayana in Bali, and University of Hasanuddin in Ujung Pandang. More than half of them (53.8%) used the Kirby-Bauer method for sensitivity testing and totally processed 383 specimens per day (Table 2). Many private laboratories performed the same test, but never exposed their results in a medical bulletin or journal.

KIRBY-BAUER METHOD

There are several methods one could use to perform the sensitivity testing, i.e. the comparison with a control method, the zone or no zone, the Kirby-Bauer and the Ericsson method. For routine testing the Department of Microbiology, Faculty of Medicine University of Indonesia uses the standardized method recommended by the NCCLS Subcommittee on Antimicrobial Susceptibility Testing which is based on Bauer *et al.*¹ It defines three degrees of sensitivity according to zone diameter: sensitive, intermediate, or resistant.

Table 2. Sensitivity testing performed in The Department of Microbiology of the Faculties of Medicine, State Universities in Indonesia 1992.

No.	Name of University	Testing method	Specimens per day
1	Syah Kuala	nr	nr
2.	North Sumatra	KB	10
3.	Andalas	nr	nr
4.	Sriwijaya	nr	nr
5.	Indonesia	KB	165
6.	Padjadjaran	KB	25
7.	Gajah mada	KB & md	97
8.	Diponegoro	nr	nr
9.	Airlangga	KB	48
10.	Brawijaya	nr	nr
11.	Udayana	KB	8
12.	Hasanuddin	KB	31
13.	Sam Ratulangi	nr	nr
Total number of specimens per day			384

Data collected during The 5th Annual Scientific Meeting of The Indonesian Society for Microbiology in Bandung on August 3-4, 1992 (KB = Kirby-Bauer; nr = no report; md = modification).

The breakpoints for each antibiotic can be obtained from the tables published by the NCCLS.²

The Kirby-Bauer method uses Mueller-Hinton agar plates of 4 mm thickness. Carefully standardized suspension of tested bacteria (turbidity comparable to a 0.5 McFarland) are inoculated on plates with a cotton wool swab to obtain a confluent growth. Not later than 15 minutes after the inoculation, antibiotic disks must be applied in order that diffusion and growth of bacteria proceed simultaneously. Press down the disks with slight pressure to ensure complete contact of the disk to the agar surface. Plates are incubated at 35°-37°C for 18 hours (overnight) in an inverted position. The diameter of each zone of inhibition is measured with a sliding caliper to the nearest millimeter. Measure the outermost rim of zone which exhibits a homogenous and complete zone of no growth if the zone of inhibition is not sharply demarcated. Referring to an interpretative table, the zone diameters for individual antibiotics are read as sensitive, intermediate or resistant.

MINIMUM INHIBITORY CONCENTRATION (MIC) AND β-LACTAMASE TEST

If minimum inhibitory concentration is required, it is determined by applying either plate dilution or tube dilution method. The latter might be performed by the macro or micro method. The ability to produce β-lactamase enzyme is detected by using cefinase paper disk or iodometric method. Both MIC determination and β-lactamase detection are not routinely performed.

REFERENCES

1. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; 45: 493-96.
2. Waitz JA et al. Performance Standards for Antimicrobial Disk Susceptibility Tests - Approved Standard, 4th ed. The National Committee for Clinical Laboratory Standards, Villanova, Pa. 1990; 10 (Number 7).

Table 2. Sensitivity testing performed in The Department of Microbiology, Faculty of Medicine, University of Indonesia, 1992

No	Name of University	Testing method	Specimens per day
1	Sriwijaya	KB	10
2	Surabaya	KB	10
3	Andalas	KB	10
4	Bekasi	KB	10
5	Bogor	KB	10
6	Brawijaya	KB	10
7	Gunung Djati	KB	10
8	Harju	KB	10
9	Indonesian Army	KB	10
10	Jember	KB	10
11	Jember	KB	10
12	Jember	KB	10
13	Jember	KB	10
14	Jember	KB	10
15	Jember	KB	10
16	Jember	KB	10
17	Jember	KB	10
18	Jember	KB	10
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38	Jember	KB	10
39	Jember	KB	10
40	Jember	KB	10
41	Jember	KB	10
42	Jember	KB	10
43	Jember	KB	10
44	Jember	KB	10
45	Jember	KB	10
46	Jember	KB	10
47	Jember	KB	10
48	Jember	KB	10
49	Jember	KB	10
50	Jember	KB	10

No	Name of University	Testing method	Specimens per day
1	Bogor	KB	10
2	Bogor	KB	10
3	Bogor	KB	10
4	Bogor	KB	10
5	Bogor	KB	10
6	Bogor	KB	10
7	Bogor	KB	10
8	Bogor	KB	10
9	Bogor	KB	10
10	Bogor	KB	10
11	Bogor	KB	10
12	Bogor	KB	10
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45	Bogor	KB	10
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50	Bogor	KB	10