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Antibiotic susceptibility of Salmonella, Shigella and Vibrio isolated from diarrhea patients in Jakarta, Indonesia

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ABSTRACT

Background: The rapid increase of antibiotic resistance among enteric pathogens in developing countries has become a great concern. In Indonesia, *Salmonella, Shigella,* and *Vibro* are still an important public health problem.

Objectives: The purpose of this study was to determine the antibiotic resistance patterns of several diarrhea-causing enteric bacteria that are frequently found in Indonesia, particularly *Salmonella, Shigella* and *Vibrio*.

Methods: A cross-sectional study was conducted , among 150 rectal swabs collected from patients with diarrhea, the enteric pathogens isolated comprised Shigella (11.4%), Salmonella (6.6%) and Vibrio (2.7%).

Results: Antibiotic susceptibility test on *Shigella* species to several antibiotics such as ampicillin, chloramphenicol, tetracycline and , trimethoprim-sulfamethoxazole showed a considerably high resistance rate (25%-100%), whereas ceftriaxone, ciprofloxacin, norfloxacin and nalidixic acid were apparently still effective (resistance rate 0%). Non-typhoid Salmonella had similar resistance patterns as those of *Shigella*, particularly to ampicillin, tetracycline, chloramphenicol and trimethoprim- sulfamethoxazole However, for *S. typhi* it was found that all antibiotics were still effective. *Vibrio* was resistant to ampicillin (resistance rate 100%), whereas the other antibiotics were still effective.

Conclusion: It may be concluded that for each of the enteric pathogens the antibiotic resistance pattern should be determined. Use of antibiotics should be based on the antibiotic susceptibility tests.

Latar Belakang: *Peningkatan pesat resistensi antibiotik antara patogen enterik di negara berkembang telah menjadi perhatian besar. Di Indonesia, Salmonella, Shigella, dan Vibro masih merupakan masalah kesehatan masyarakat yang penting.*

Tujuan: *Tujuan dari penelitian ini adalah untuk menentukan pola resistensi antibiotik dari beberapa bakteri enterik penyebab diare yang sering ditemukan di Indonesia, khususnya Salmonella, Shigella dan Vibrio.*

Metode: *Sebuah studi* cross-sectional *dilakukan, antara 150 penyeka dubur yang dikumpulkan dari*

pasien dengan diare, patogen enterik terisolasi terdiri Shigella (11,4%), Salmonella (6,6%) dan Vibrio (2,7%).

Hasil: *Uji kepekaan antibiotik pada spesies Shigella beberapa antibiotik seperti ampisilin, kloramfenikol, tetrasiklin dan, trimethoprim-sulfamethoxazole menunjukkan tingkat resistensi yang cukup tinggi (25% -100%), sedangkan ceftriaxone, ciprofloxacin, norfloksasin dan asam nalidiksat yang tampaknya masih efektif (resistensi tingkat 0%). Salmonella non-tifoid memiliki pola resistensi yang sama seperti orang-orang dari Shigella, khususnya terhadap ampisilin, tetrasiklin, kloramfenikol dan trimethoprim-sulfametoksazol Namun, untuk S. typhi ditemukan bahwa semua antibiotik masih efektif. Vibrio adalah resisten terhadap ampisilin (tingkat resistensi 100%), sedangkan antibiotik lain yang masih efektif.*

Kesimpulan: *Dapat disimpulkan bahwa untuk setiap enterik yang patogen pola resistensi antibiotik harus ditentukan. Penggunaan antibiotik harus didasarkan pada uji kepekaan antibiotik.*

INTRODUCTION

Antibiotic treatment against bacterial infections is generally an important factor in reducing the morbidity and mortality rates. However, excessive and incorrect use of antibiotics for the treatment of disease may increase the occurrence of bacterial resistance to these antibiotics.[1,2] This results in reduced benefits and efficacy of antibiotics commonly used in therapy, thus causing serious problems, such as a longer course of the infectious disease and unnecessary exposure of the patient to drug toxicity.

Antibiotic resistance may cause inappropriate selection of antibiotics at the start of treatment, as antibiotic administration is done empirically. The results of the treatment are unsatisfactory due to of the ineffectiveness of the antibiotic against already resistant bacteria. This problem becomes important because of increased prevalence of resistance againts antibiotic drugs commonly used in the treatment of infectious disease. The development of antibiotic resistance is accelerated by the use of antibiotics in animals and their inappropriate use in humans.[3] The rapid and continuous occurrence of antibiotic resistance in enteric pathogenic bacteria to first-line antibiotics for the treatment of diarrhea has caused various problems and concerns in developing countries.[1,4]

In developing countries the diarrhea-causing enteric pathogenic bacteria, such as *Salmonella*, *Shigella* and *Vibrio,* still constitute an important public health problem.[4] Although *Salmonella* infections are self-limiting, i.e. the patient recovers after a time without specific treatment, the disease may be severe and fatal, so that antibiotics are needed. [5-7] In addition, there are reports from various countries of increased resistance rates and the occurrence of multiresistance in *Salmonella*.[8] In cases of infections caused by Shigella, high mortality rates have been reported as well as malnutrition and growth disturbances in children as a result of the disease.[9] Shigellosis is related to poor hygiene, poverty and crowded housing. In the last few decades *Shigella* species have shown changes in antibiotic susceptibility patterns. These bacteria progressively show resistance to various antibiotics primarily used in the treatment of diarrhea.[10] They are resistant to conventional antibiotics such as ampicillin, tetracycline, and trimethoprim-sulfamethoxazole but generally are sensitive to fluoroquinolones.[9]

For cholera, antibiotic treatment is important because it may reduce the frequency of diarrhea and the volume of stools excreted by the patient.[8] For more than 30 years tetracycline had been the drug of choice for the treatment of cholera. To date no significant numbers of *Vibrio* cholerae resistant to this antibiotic have been found in Indonesia. In contrast, increased resistance rates to several antibiotics have been found in *V. parahaemolyticus*.[9] For patients with diarrhea in developing countries, laboratory investigations, comprising bacterial culture and antibiotic resistance test, are rarely performed, because of the high cost of the investigations. Patients usually receive antibiotic treatment on the basis of clinical signs and symptoms of diarrhea without laboratory confirmation.

The purpose of this study was to determine the antibiotic resistance patterns of several

diarrhea-causing enteric bacteria that are frequently found in Indonesia, particularly *Salmonella, Shigella* and *Vibrio*. Understanding the antibiotic resistance patterns of these pathogens is important, because it may guide empirical treatment in the framework of reducing inapproriate antibiotic use.

METHODS

Study subjects and location. This study was conducted on patients with diarrhea who attended Community Health Center, South Jakarta, Indonesia, from September 2011 until May 2013. Diarrhea was defined as three or more loose stools during the previous 24 hours.[11]

Informed consent and patient information. Before sample collection, signed informed consent for voluntary participation in the study was requested from the patient or guardian (in case of a child). After obtaining consent, a clinical questionnaire was completely filled out by study personel. Information on personal data and the disease of the patient was recorded by health personel appointed for this task, followed by collection of a stool specimen. The data collected from the subjects comprised age, gender, clinical signs and symptoms, and stool samples or rectal swabs for bacterial culture and antibiotic susceptibility testing

Bacteriological procedures

Media.

The culture media used in this study consisted of xylose-lysine-deoxycholate agar (XLD), MacConkey agar (MAC), *Salmonella-Shigella* agar (SS), and thiosulfate citrate bile salts sucrose agar (TCBS). All media were obtained from DIFCO, Becton Dickinson, Sparks, MD, and prepared according to previously described methods [12,13]

Study material and culture process

Rectal swab was collected from patient with diarrhea who attended the Community Health Center. The sample was taken irrespective of disease severity (mild, moderate, or severe). Rectal swab was collected at the time of attendance and before administration of antibiotics. Care was taken to collect the swab before the fourth day of illness, since after four days the bacterial population would have decreased, presumably resulting in a false negative. Rectal swab was placed in Cary Blair transport medium and stored in a refrigerator until transported to the laboratory. The samples were transported under cold conditions by placing them in a thermos bottle. On arrival at the laboratory the rectal swabs were plated onto MAC, SS, XLD and TCBS. The agar plates were incubated aerobically at 37°C for 18-24 hours. Suspected colonies were picked and grown in biochemical media for identification and characterization.[12,13] Serological tests for confirmation were done on each bacterial species isolated using specific antiserum (DIFCO, Becton Dickinson, Sparks, MD).

Antibiotic susceptibility testing

Bacterial isolates were subsequently subjected to antibiotic susceptibility testing against ampicillin (Am), chloramphenicol (C), tetracycline (Te), trimethoprim sulfamethoxazole (Sxt), ceftriaxone (Cro), ciprofloxacin (Cip), norfloxacin (Nor) and nalidixic acid (Na), The test was performed using the disk diffusion method in accordance with the guidelines of Clinical and Laboratoy Standards Institute.[14] The antibiotic disks used in this test were obtained from Becton Dickinson and Company, Cockeysville, MA, USA. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were included in the test as control strains. The test result was read by measuring the inhibition zone demonstrated by the culture. Determination of sensitive, resistant or intermediate was in accordance with established standards. [14]

RESULTS

Overall, 150 rectal swab specimens were collected from patients with diarrhea of all ages without regard to disease severity. From these rectal swabs the isolated enteric pathogenic bacteria consisted of *Shigella* (11.4%), *Salmonella* (6.6%) and *Vibrio* (2.7%) species. *Shigella* strains isolated were *S. dysenteriae* (0.7%), *S. flexneri* (6.7%), *S. sonnei* (2.7%) and *S. boydii* (1.3%). *Salmonella* sp isolated consisted of non-typhoid *Salmonella* and *S. typhi*, with percentages of 5.3% and 1.3%, respectively. The remaining

isolates were species of *Vibrio*, namely *Vibrio* non-O1 (2.0%) and *V. parahaemolyticus* (0.7%) (Table 1).

Results of suceptibility test showed variable resistance among the various bacterial isolates. Of the *Shigella* species, *S. flexneri* showed the highest resistance rate (80%-90%) to ampicillin, chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole. S. sonnei and S. boydii were mostly resistant to tetracycline and trimethoprim-sulfamethoxazole (75%-100%), while all *S. dysenteriae* tested (100%) showed resistance to chloramphenicol and trimethoprim-sulfamethoxazole (Table 1). For non-typhoid *Salmonella*, resistance to ampicillin and tetracycline comprised more than half (62.5%) of the isolates, however many were still sensitive to chloramphenicol and trimethoprim-sulfamethoxazole (75% and 87.5%, respectively). For *S.typhi* no isolates were encountered that were resistant to the antibiotics tested. *Vibrio* cholera was not found in this study, but of the *Vibrio* non-O1 and *V. parahaemolyticus* that were isolated, all were resistant to ampicillin (100%) but sensitive to the other antibiotics.

DISCUSSION

Resistance to antibiotics may result in changes in virulence of microorganisms and poor response to empirical antibiotic treatment, because the microorganisms are already resistant to the antibiotic in question. Infections caused by antibiotic-resistant microorganisms tend to lead to a longer or a more severe course of the disease, in comparison with infections by bacteria that are still sensitive to antibiotics.

In recent years an increased incidence of infections have been reported, and also multiantibiotic resistance by non-typhoid *Salmonella.* [15,16] They stated on the basis of their study results that of all isolated non-typhoid *Salmonella* serotypes, *S. Newport* was the most resistant to tetracycline, although it was still sensitive to the other antibiotics tested. The majority of the multiresistant serotypes were *S. Typhimurium* and *S. Hadar*. Overall, among the isolated and tested non-typhoid *Salmonella* species in the study of Bukitwetan et al[16] many were multiresistant, as had also been reported by other investigators.[15] In our study, the non-typhoid *Salmonella* species obtained were on average resistant to four first-line antibiotics that are commonly used in therapy and 20% of these were even resistant to nalidixic acid (Table 1). However, only a small percentage of the non-typhoid Salmonella were resistant to chloramphenicol, an antibiotic that had previously been the drug of choice for *Salmonella* infections, particularly *S. typhi*.

S. typhi species which we isolated did not show resistance to the eight antibiotics tested, as they were sensitive to all. However, because of the extremely small number of isolates that we obtained, viz. only two (1.3%), this does not constitute a general picture of the antibiotic sensitivity status of S. typhi in Jakarta. On the other hand, the report of Ochiai et al[17] stated that in Indonesia, with an *S. typhi* isolation rate of 3.31%, no antibiotic-resistant *S. typhi* was found, as is the case with China. This situation is very different from that of *S. typhi* from Vietnam, Pakistan, and India, where many *S. typhi* isolates are already resistant to antibiotics, and even show multidrug resistance (MDR), i.e. to ampicillin, chloramphenicol and trimethropim-sulfamethoxazole, the percentages being 7% in India and up to 65% in Pakistan17. The presence of MDR accompanied by resistance to fluoroquinolones and nalidixic acid, which is developing rapidly in Vietnam, results in problems and difficulties in the treatment of typhoid fever. In 2004 it was reported that isolates of *S. typhi* in Vietnam, which in the previous five years had a resistance rate of 87% to nalidixic acid, in that year had a resistance rate of up to 97% 18. Patients with S. typhi infections resistant to nalidixic acid show a poor clinical response with a therapeutic failure rate of up to 36% accompanied by a prolonged fecal carrier state. Although in South Vietnam MDR in S. typhi has been reportedly high in the last 13 years or more, there are reports that in other regions isolates of *S. typhi* are encountered that are again sensitive to chloramphenicol, although in general in other Asian countries the MDR rate of *S. typhi* is still high. [17-19]

During the last few years no cholera epidemic has been reported in Indonesia and

the prevalence of cholera is not high, namely around 3%. In spite of that, cholera, which causes acute diarrhea, is still considered to be a health hazard in the under-fives, particularly babies younger than 1 year of age 20 . In our present study no *V. cholerae* O1 was found. This may have been due to an inadequate sample size that did not allow the isolation of *V. cholerae* O1, whose current incidence tends to be low, namely only 0.5/1000/year 20. Other isolated *Vibrio* species were *V. cholerae* non-O1 (2.0%) and *V. parahaemolyticus* (0.7%). Both *Vibrio* species are in general still sensitive to all tested antibiotics, except ampicillin, to which they were fully resistant (100%). Agtini et al[20] reported that in a survey conducted in Jakarta, >90% of isolates of *V. cholerae* O1 were still sensitive to trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol, besides fluoroquinolones. However, although they successfully isolated non-cholera bacteria, Agtini et al [20] in their reports did not mention the antibiotic sensitivity patterns of these bacterial species. Antibiotic resistance of *V. cholerae* non-O1 and V. parahaemolyticus was reported by Tjaniadi et al [21]. It was stated that antibiotic resistance in *V. cholerae* non-O1 was from year to year clearly increasing in frequency with regard to antibiotics commonly used in the treatment of diarrhea in Indonesia. According to the report [21], *V. parahaemolyticus* also showed an increasing frequency of antibiotic resistance, although its pattern was slightly different from that found in *V. cholerae* non-O1. *V. parahaemolyticus* was still sensitive to most of the antibiotics, except for a few number (3-15%) that were resistant to tetracycline and chloramphenicol, while to ampicillin all isolates (100%) were already resistant. This situation is similar to that found in our study, so that it may be stated that in the last 10 years there have not been many changes in the antibiotic susceptibility pattern of *V. parahaemolyticus*.

Shigella is the main cause of diarrhea leading to high mortality in developing countries and consistently associated with the syndrome of clinical dysentery, prolonged diarrheal episodes and persistent diarrhea.[9] Episodes of dysentery are also a risk factor for malnutrition and growth disturbances in children. Antibiotic resistance of these bacteria is developing progressively in Asian, African and Latin American countries.[9] The study of Kosek et al [9] reported that overall the isolates of *Shigella* obtained (63%-83%) were already resistant to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline and erythromycin, but were in general still sensitive to quinolones and ceftriaxon. Similar antibiotic resistance patterns of Shigella have also been reported by other investigators.[21-23]

In our study, among the isolates of *Shigella* that we obtained and of which we tested the antibiotic sensitivity, all showed resistance to the main antibiotics, i.e. ampicillin, chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole. However, different serotypes had different sensitivity patterns. All *Shigella* serotypes were already resistant to trimethoprim-sulfamethoxazole except for *S. flexneri* (90% resistant), the other serotypes, namely *S. dysenteriae, S, boydii* and *S. sonnei*, were all (100%) resistant to trimethoprim-sulfamethoxazole (Table 1). Of the four serotypes that could be isolated, *S. flexneri* and *S. sonnei* showed multi-antibiotic resistance, namely to ampicillin, chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole. To other antibiotics such as the quinolones ciprofloxacin, norfloxacin, nalidixic acid, and also to ceftriaxone, all *Shigella* isolates were still sensitive.

The survey conducted by Agtini et al[20] in North Jakarta reported that more than 73% isolates of *S. flexneri* were resistant to the four antibiotics as in our study. Apparently during the last few years there have not been many changes in the antibiotic sensitivity patterns of *S. flexneri* and other *Shigella* species in Jakarta. Because of the high frequency of antibiotic resistance of *Shigella* species to those four antibiotics, they cannot be used anymore for the treatment of shigellosis. Although there are recommendations to give antibiotic treatment to shigellosis in children, the main concern is the selection of antibiotics to be given in the treatment, in view of the great number of resistant *Shigella* species. The use of fluoroquinolones in children with shigellosis is still controversial, and therefore alternative drugs should be looked for. According to the report of Herwana et al [24] and our own study

results, no resistance of *Shigella* to nalidixic acid has been found, so that nalidixic acid is currently the antibiotic of choice in the treatment of shigellosis in children in Jakarta or even in Indonesia. Except for various reports on the resistance of *Shigella* species, from our study results it is difficult to come to a conclusion about the antibiotics susceptibility of *S. dysenteriae* and *S. boydii*, because of the small numbers of isolates that we obtained. For *Vibrio* species there are not many problems in antibiotic treatment, however those antibiotics that a few years ago were in common use for the treatment of shigellosis and salmonellosis, are currently of no benefit, because of the high frequency of resistance in these bacteria.

Although the mechanisms of resistance in organisms have been clearly known, including selective pressure as a result of exposure to antibiotics, the precise role of drug usage in the selection of antibiotic resistance cannot be fully explained yet. Therefore, it is recommended to be careful in the use of antibiotics for the treatment of infectious disease.

CONCLUSION

For each of the enteric pathogens the antibiotic resistance pattern should be determined. Understanding the antibiotic resistance patterns of these pathogens is important, because it may guide empirical treatment in the framework of reducing inapproriate antibiotic use. Use of antibiotics should be based on the antibiotic susceptibility tests.

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