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BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF SURGICAL SITE INFECTIONS AT A TERTIARY CARE CENTRE IN SOUTH KERALA

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ABSTRACT

Background: Among health care associated infections, Surgical site infections (SSIs) constitute to be a major concern, by increasing hospital cost, net morbidity and mortality related to surgical interventions. The etiological agents pertaining to these infections vary from hospital to hospital, depending on the endemic flora in a system.

Methodology: A prospective study on clean & clean-contaminated post operative wounds of 280 patients at Sree Gokulam Medical College in one year was conducted, to study the prevalence rate of surgical site infections, the bacteriological profile of organisms and their susceptibility pattern. A minimum of two samples were collected, one at the time of the first dressing and the second in evidence of any infection; for bacteriological culture and sensitivity. All data was analysed by SPSS software.

Results: The SSI rate was 7.5% with 5.4% in clean and 31.8% in clean-contaminated wounds. Staphylococcus aureus (61.8%; with 66.7% MRSA) was most frequently isolated, followed by Escherichia coli (14.3%). Others were Enterobacter cloacae (9.5%), Pseudomonas aeruginosa (4.8%), Klebsiella pneumoniae (4.8%) and Streptococcus agalactiae (4.8%).

Conclusion: The infection rate amongst clean and clean-contaminated surgeries was 7.5%, which required intervention. The most frequently isolated strain was Staphylococcus aureus (61.8%) with clear predominance of MRSA. The gram-negative isolates were mostly multi-drug resistant, with predominance in. members of Enetrobacteriaceae.

Keywords: Surgical site infection, Healthcare associated infection, Clean and clean-contaminated wounds, Staphylococcus aureus, Standard Antimicrobial Prophylaxis.

INTRODUCTION

The healthcare system of the present era has met with a lot of challenges in controlling surgical site infections. In spite of coming a long way in the field of infection control, these infections have persisted as a serious issue to both the surgeon as well as the microbiologist. According to CDC of the US, A surgical site infection simply refers to an infection that occurs after surgery in the part of the body where the surgery took place. The ECDC (European Centre for Disease Prevention & Control) defines as it an infection that occurs within 30 days after the operation and involves the skin and subcutaneous tissue of the incision (superficial incisional) and/or the deep soft tissue (for example, fascia, muscle) of the incision (deep incisional) and/or any part of the anatomy (for example, organs and spaces) other than the incision that was opened or manipulated during an operation (organ/space)[1]. Though the Centre for Disease Control and Prevention (CDC) reports give an update of surgical site infections, being 22% of the total HAI,[2] the real issue is of a much larger magnitude. This is due to the fact that most cases of SSI manifest after discharge, and is missed out in surveillance. Studies show that SSIs were the most common healthcare-associated infection, accounting for 31% of all HAIs among hospitalized patients[3].

Post-operative wound infections manifest in at least 2% of all hospitalized patients undergoing surgical procedures ranging from 2.5% to 41.9% globally, resulting in high morbidity and mortality[4,5]. Approximately 2% to 5% of the 16 million people undergoing surgical procedures each year develop surgical site infection with more recent data putting it at two-thirds of patients who undergo operations.[6,7] Undoubtedly, it is the most expensive HAI type; it has an estimated cost of 3.3 billion dollars globally, with nearly 1 million additional inpatient days annually. Each patient with SSI stays an additional 7 – 11 days in the hospital and has 2 – 11 times the higher death risk than other post-operative patients. Besides, it is the most frequent cause of unplanned readmissions after a surgical procedure. While the CDC NHSN data from the US reports a 17% reduction of SSI rates related to 10 selective procedures between 2008 and 2014, several multicentric studies from India point to an SSI rate ranging from 4.1% to 11.0%. The true data is expected to be much higher as the post-discharger follow up is a big challenge in SSI surveillance.[7-9]

These infections are usually caused by either exogenous or endogenous micro-organisms, or both[10,11]. They enter the operative wound either during the surgery (primary infection) or after the surgery (secondary infection). However, the period of greatest risk remains the time between opening and closing the operating site[12]. Primary infections are more serious, appearing within five to seven days of surgery. Most of these infections appear between the 5th and 10th day after operation, and can be reduced by the appropriate use of surgical antimicrobial prophylaxis[13]. In hospital practice 30- 50% of antibiotics are prescribed for surgical prophylaxis and 30-90% of this prophylaxis is inappropriate. This inappropriate use increases the selection pressure, favouring the emergence of pathogenic drug resistant bacteria, hence increasing the risk of post-operative wound infections[14].

In most cases of SSIs, the causative pathogens originate from endogenous flora of the patient's skin, mucous membranes or hollow viscera[15]. The microbiology of a post-operative wound generally depends on the nature of surgery, the site of incision and the body cavity/hollow viscous entered during the procedure. Among these organisms, the gram-positive bacteria such as the *Staphylococcus species* (both coagulase positive and negative isolates) are mostly involved in clean surgeries while *Enterococcus* species in contaminated wounds. The gram-negative organisms such as *E. coli, Klebsiella pneumonia, Proteus* spp, *Pseudomonas species and Acinetobacter species* predominate the contaminated surgeries., as quoted from various studies[14,16-19, 20].

MATERIALS & METHODS

A prospective study was conducted among the patients who underwent surgery at Sree Gokulam Medical College in the departments of Surgery, Orthopaedics, Obstetrics & Gynaecology, and Gastro-surgery. The surgeries were classified into clean, clean-contaminated, contaminated and dirty according to various guidelines[21-24]. The study population belonged to patients undergoing clean & clean-contaminated surgeries only. Contaminated & dirty surgeries, cases of stitch abscesses, post burns patients, and Infection presenting after the period of surveillance as per CDC criteria were excluded. The study was conducted in 1 year. (Jan 1st 2017 – Dec 31st 2017). The sample size was calculated to 280 by the formula $z_{\alpha}^{2}pq/d^{2}$ where p standard deviate = 16. SSI was diagnosed as per the guidelines issued by the Centre for Disease Prevention and Control.

A minimum of 2 samples were collected from the surgical site by cotton swabs. Tissue and Aspirated pus samples were preferred over swabs wherever possible. the first sample was taken during the first dressing and subsequent swabs only in case of any evidence of infection such as fever, signs of inflammation, wound gaping, or discharge. The wound was thoroughly washed with saline to remove any visible debris & loose necrotic tissue. No betadine or antiseptic solution was used before swabbing. The area selected was the highly vascular granulation tissue, rather than the yellow fibrous slough or pooled exudates. The material was collected by pressing the swab over the clean wound site to extract tissue fluid; the most probable site for location of the potential pathogen. The double swabs were processed by routine microbiological methods. Gram smear was prepared and examined for the presence of pus cells and to observe the morphology of any organism if present. The swab collected for culture and sensitivity was plated on blood agar, MacConkey agar and mannitol Salt Agar. The culture plates were incubated at 37 degrees for 24 hours and examined for growth. Colony smears were prepared from it (after noting the colony characteristics) and was subjected to biochemical identification using standard biochemical tests and further tested for antibiotic sensitivity. If the plates showed no growth, the plates were further incubated for another 24 hours before declaring culture sterile. Antibiotic susceptibility was studied by Kirby Bauer's disc diffusion method on Muller Hinton Agar, as per CLSI guidelines. The antibiotic panel of testing was decided on basis of the culture smear obtained on gram staining. For gram positive organisms, the following antibiotics were used: penicillin, cefoxitin, amoxycillin clavulanate, cefalexin, erythromycin, clindamycin, cotrimoxazole, tetracycline, gentamicin, netilmicin, amikacin, vancomycin, teicoplanin, rifampicin and linezolid. For gram negative organisms, ampicillin, amoxycillin clavulanate, cefalexin, cefuroxime, cefotaxime, cefepime, gentamicin, netilmicin, amikacin, ciprofloxacin, ofloxacin, aztreonam, cotrimoxazole, tetracycline, imipenem, meropenem, piperacillin, piperacillin tazobactam, cefaperazone sulbactam, tigecycline, colistin and cefoxitin were tested.

Staphylococcus aureus strains isolated from infected surgical wounds were screened phenotypically for MRSA (methicillin resistant staphylococcus aureus) using cefoxitin 30g as a surrogate marker. Gram-negative isolates were screened for the production of ESBL (extended spectrum beta lactamase) and CR (carbapenamase) enzymes, using Disc Diffusion test and Modified Hodge Test respectively. All data were evaluated by chi square test (X^2 statistical test. $P \leq 0.05$ was considered to be significant) using SPSS software.

RESULTS

Out of the 280 patients, 21 patients showed clinical signs of SSI with positive growth on culture, (7.5%). 259 patients (92.5%) showed no growth. Among the 21 cases, *Staphylococcus aureus* was the most commonly isolated organism (61.8%). The others were *Escherichia coli* (14.3%), *Enterobacter cloacae* (9.5%), *Pseudomonas aeruginosa* (4.8%), *Klebsiella pneumoniae* (4.8%), and Group B Streptococcus (4.8%). *S. aureus* was the predominant isolate in both clean and clean-contaminated classes of wounds. In this study, more isolates were obtained in clean wounds (69.2%). 66.7% of *S. aureus* obtained were MRSA.

The gram-negative isolates were mostly multi-drug resistant, and were 100% resistant to 1st, 2nd and 3rd generations of cephalosporins except for *Enterobacter cloacae* which showed 50% susceptibility. *Pseudomonas aeruginosa* showed 100% susceptibility to all aminoglycosides. All *E. coli* isolates showed 100% susceptibility to beta lactam-beta lactamase inhibitors, (except for amoxycillin clavulanate – 100% resistant) and carbapenems.

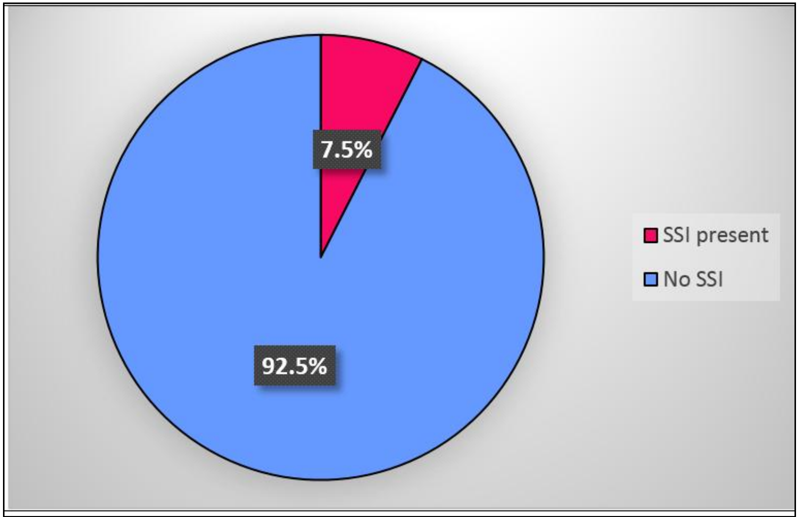

Figure 1: Rate of Surgical site Infection (SSI)

Of the total 280 patients, 21 patients showed growth on culture, the SSI rate being 7.5%. 259 patients (92.5%) showed no growth on culture.

Organism Isolated	**Frequency**	**Percentage**
Staphylococcus aureus	13	61.8
Pseudomonas aeruginosa	1	4.8
E. coli	3	14.3
Klebsiella pneumoniae	1	4.8
Enterobacter cloacae	2	9.5
GBS	1	4.8
Total	21	100

Table 1: Distribution of Isolates Obtained in Culture

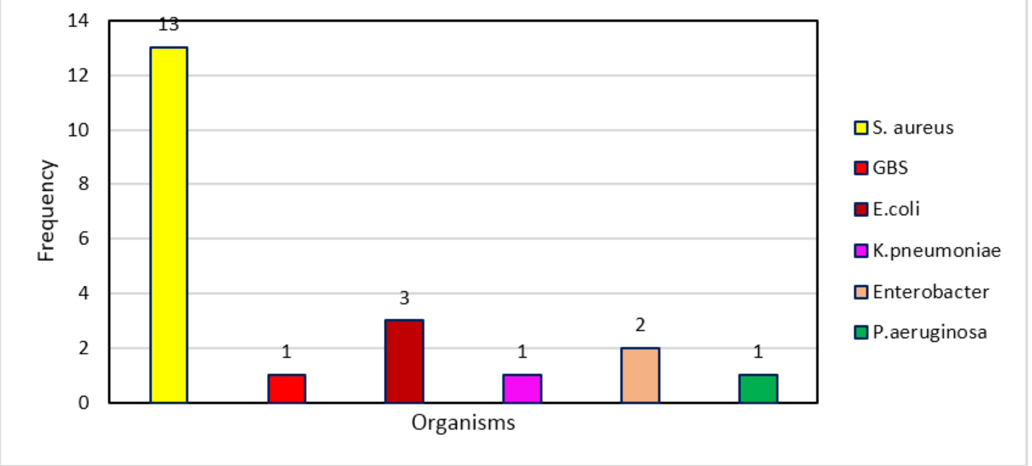

Figure 2: Distribution of Isolates Obtained in Culture

Of the 21 cases that developed infection, *Staphylococcus aureus* was the most common isolate (61.8%). The other organisms obtained were *E. coli* (14.3%), *Enterobacter cloacae* (9.5%), *Pseudomonas aeruginosa* (4.8%), *Klebsiella pneumoniae* (4.8%), and Group B Streptococcus (4.8%).

Serial No	Isolate	Clean		Clean-Contaminated		Total	
		Frequency	%	Frequency	%	Frequency	%
1	S. aureus	9	69.2	4	57.1	13	61.9
2	P. aeruginosa	1	7.1	0	0.0	1	4.8
3	E. coli	3	21.4	0	0.0	3	14.3
4	K. pneumoniae	0	0.0	1	14.3	1	4.8
5	E. cloacae	0	0.0	2	28.6	2	9.5
6	Group B Streptococcus (GBS)	1	7.1	0	0.0	1	4.8
	Total SSI	14	100	7	100	21	100

Table 2: Type of Isolates Obtained in Clean and Clean-Contaminated Surgeries

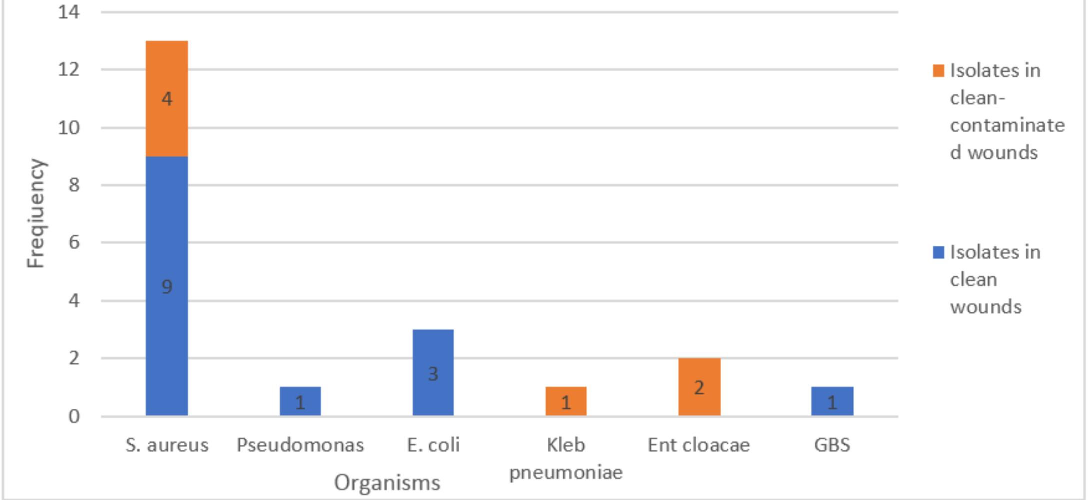

Figure 3: Type of Isolates Obtained in Clean and Clean-Contaminated Surgeries

While S. aureus was the predominant isolates in both classes of wounds, more no of isolates was obtained in clean wounds (69.2%).

Antibiotic Prophylaxis	SSI				Total	%	Organisms isolated
	Present	%	Absent	%			
Cefotaxime	13	6.9	175	93.1	188	100	S. aureus, P. aeruginosa E. coli, Ent. cloacae
Cefalexin	1	20.0	4	80.0	5	100	S. aureus
Ceftriaxone	5	16.7	25	83.3	30	100	S. aureus, K. pneumoniae, Group B Streptococcus
Amoxycillin clavulanate (AC)	Nil	-	15	100	15	100	Nil
Cefaperazone Sulbactam (CS)	2	8.0	23	92.0	25	100	S. aureus, E. coli
Cefotaxime+ Metronidazole (TxM)	Nil	-	6	100	6	100	Nil
CS+ Metronidazole (CSM)	Nil	-	11	100	11	100	Nil
Total	21	7.5	259	92.5	280	100	

Table 3: Correlation of the Rate of SSI and Antibioitc Prophyllaxis

DISCUSSION

Surgical site infections (SSI), a major part of hospital acquired infections (HAI), constitute about 20 – 25% of HAI.[18] In this study the various isolates obtained the rate of surgical site infections were studied through different variables such as gender of the patient, type of surgery, duration of the surgical procedure, duration of the period of post-operative hospital stay, associated comorbidities (diabetes mellitus, steroid therapy, malignancy and HIV infection), the type of wound (clean / clean-contaminated) nature of surgery (elective / emergency), and the antibiotics administered both preoperatively and postoperatively.

The overall rate of surgical site infection obtained was 7.5%; On analysing the bacteriological profile, both gram positive and gram-negative organisms were isolated. Gram positive organisms constituted the major part of the isolates (66.7%), in contrast to gram negative spectrum (33.3%). Among the gram-positive organisms were *Staphylococcus aureus* (61.8%) and *Streptococcus agalactiae* or Group B Streptococcus (4.8%). The gram-negative isolates included *Escherichia coli* (14.3%), *Enterobacter cloacae* (9.6%), *Klebsiella pneumoniae* (4.8%) and *Pseudomonas aeruginosa* (4.8%). The major isolate obtained in the study was *Staphylococcus aureus* in both clean and clean-contaminated cases. (In infected clean surgeries, *S. aureus* was isolated in 69.2% cases while the infected clean-contaminated cases gave 57.1% isolation. This can be attributed to the fact that *S. aureus* is a common skin and nasal coloniser and is reported to infect clean surgeries more.) Similar results have been obtained in various studies; isolating *S. aureus* as the chief pathogen, such as those by Gupta et al,[25] P. Preetishree et al,[14]Negi et al,[26] Subrata Roy et al,[27]Lilani et al,[5]Anvikar et al,[6] Kumar Ansul et al[28] and Patnaik et al[29] The other isolates in clean surgeries was *E. coli* (14.3%), *Pseudomonas aeruginosa* (4.8%), and Group B Streptococcus (4.8%); while in clean-contaminated cases, the other organisms obtained were *Enterobacter cloacae* (9.5%), and *Klebsiella pneumoniae* (4.8%). No mixed isolates were obtained, as contaminated wounds were completely avoided, and all swabs were taken after thorough saline washing.

As mentioned, *S. aureus* was the most frequently obtained isolate (61.8%). In order to study methicillin resistance, cefoxitin disc diffusion method with cefoxitin 30μg disc as a surrogate marker was used as per CLSI guidelines. 66.7% were detected as *Methicillin Resistant Staphylococcus aureus* (MRSA). High rate of MRSA in SSI has been reported by numerous authors: Prasanna Gupta et al[25] (83%), P. Preetishree et al[14] (77.8%), Khyadi Jain et al[30] (48.78%) and Neha Patnaik et al[29] (52.38%). No VRSA was isolated. Inducible clindamycin resistance was assessed by D test. 75% of the MRSA isolates were D test positive. The relative risk of surgical site infections is 2-9 times greater in carriers of *Staphylococcus aureus* than in non-carriers. The only gram-positive isolate obtained other than S. aureus, was an isolate of Group B Streptococcus. The biochemical identification was done by a positive CAMP test and confirmation by latex agglutination with antisera. It is a rare isolate in context to SSI, and was obtained from the mastectomy site of an elderly male patient diagnosed with carcinoma breast and diabetes mellitus. In a study by P J Jenkins et al,[31] there has been reported incidence of Group B Streptococcus in surgical site infections. But it has been linked to immunocompromised patients only.

The Gram-negative isolates obtained were all Enterobacteriaceae (E. coli (14.3%), Enterobacter cloacae (9.5%) and Klebsiella pneumoniae (4.8%)), except for one; Pseudomonas aeruginosa (4.8%). Majority of the isolates obtained were multi-drug resistant (MDR), with high degree of resistance to base line drugs such as cephalosporins and quinolones.

From the obtained *E. coli* isolates, 66.7% were MDR pathogens. 33.3% were ESBL producers. Ampicillin, and all classes of Cephalosporins tested; (from class I to class IV) showed 100% resistance. 33.3% susceptibility was shown to first generation aminoglycosides such as gentamicin and first-generation quinolones such as ciprofloxacin, in addition to cotrimoxazole and tetracyclines. The sensitivity to second-generation aminoglycosides and quinolones was better; with 66.7% and 100% susceptibility to ofloxacin and amikacin respectively. 66.7% isolates were sensitive to beta-beta lactamase inhibitors such as cefaperazone sulbactam and piperacillin tazobactam. On testing carbapenems, imipenem showed 66.7% sensitivity while meropenem had 100% sensitivity. Colistin showed 100% susceptibility to all isolates.

Klebsiella pneumonia obtained was an MDR isolate. Ampicillin, all beta-beta lactamase inhibitors tested (Amoxycillin Clavulanate, Cefaperazone Sulbactam and Piperacillin Tazobactam), Fluroquinolones and Carbapenems exhibited 100% resistance. The resistance pattern exhibited indicates that multiple drug resistance mechanisms may have come into play. Aminoglycosides, Cefoxitin and Colistin showed 100% susceptibility.

Enterobacter cloacae was isolated only from arthroplasty cases; all clean-contaminated. Quinolones, Carbapenems, Amikacin, Cefaperazone Sulbactam, Piperacillin Tazobactam and Colistin showed 100% susceptibility. Amoxycillin clavulanate, Cephalosporins, Cotrimoxazole, Gentamicin, and Tetracycline showed 50% susceptibility. Ampicillin showed 100% resistance.

Pseudomonas aeruginosa was isolated from a case of umbilical hernia repair. It was also an MDR isolate; Ampicillin, Piperacillin, Cephalosporins, Quinolones, Cotrimoxazole and Carbapenems showed 0% susceptibility while Aminoglycosides (including gentamicin, amikacin, and tobramycin) Cefaperazone Sulbactam, Piperacillin Tazobactam and Colistin exhibited 100% susceptibility.

On a broad basis, the gram-negative isolates obtained showed higher susceptibility to aminoglycosides, carbapenems, bet lactam-beta lactamase inhibitors and colistin, while most isolates were resistant to cephalosporins, quinolones and sulphonamides. This was similar to other studies. In study by Shahana et al.[32] Enterobacteriaceae showed the highest sensitivity to amikacin (78%) followed by gentamicin (71%) and very low sensitivity was noted with the cephalosporins and fluoroquinolones (10% and 58% respectively). In another study by S. Madhavi et al, it was found that almost all the isolates were mostly sensitive to Amikacin – Pseudomonas species were mostly sensitive to Amikacin (82.4%) followed by Ofloxacin (76.4%), *Escherichia coli* isolates were 100% sensitive to Amikacin and Klebsiella species were sensitive to both Amikacin and Gentamicin (90.9%). Similar findings were observed in yet another study by Bansal et al,[33] where all the gram-negative isolates showed resistance to penicillin, ampicillin and amoxycillin clavulanate (100%) and most of the isolates showed resistance to cephalosporin group (>70%) and quinolones (70%). Also, a few isolates showed sensitivity to a combination of cefaperazone-sulbactam (42%). Meropenem was found to be effective against 50-53% isolates and 42-57% of the gram-negative organisms showed sensitivity against aminoglycoside drugs such as amikacin but to a lesser extent with gentamycin (26%). The isolates were screened for ESBL and carbapenamase production by disk diffusion method and Modified Hodge test respectively, according to CLSI guidelines. 33.3% of E. coli isolates were ESBL producers. No other positive results were obtained. This pattern of drug resistance in the gram-negative organisms can be attributed to other non-enzymatic resistance mechanisms such as production of efflux pumps, reduced membrane permeability, ribosomal inactivation etc.

CONCLUSION

In spite of the advances in managing surgical wounds and antimicrobial stewardship, SSIs still pose a major challenge in the health care system. As we know, Surgical site infections cannot be completely eliminated. But regular surveillance measures as well as effective antibiotic policies can create a significant difference in this scenario.

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