Analysis of antibiotic usage for surgical prophylaxis in a tertiary care hospital in Bangalore, Karnataka, India

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Introduction: Appropriately administered antibiotic prophylaxis reduces the incidence of surgical wound infection. The timing of antibiotic administration is very critical. The first dose should always be given before the procedure, preferably within 60 minutes before incision. Re-administration at one to two half-lives of the antibiotic is recommended for the duration of the procedure. In general, postoperative administration is not recommended except for cardiothoracic surgeries.

Materials and Methods: This is a retrospective study done over a period of 6 months from January 2019 to June 2019 in a tertiary care hospital in Bangalore. A continuous 100 patients who underwent an elective surgery were audited regarding the antibiotic indication, choice, dosage, dosing interval, and timing of first antibiotic administration prior to skin incision and duration of prophylaxis were compared with the CDC guideline recommendations and hospital antibiotic policy.

Results: A total of 100 surgeries were audited. Out of this, 4% were clean 84% were clean contaminated 4% were contaminated and 8% were dirty. The most commonly used antibiotics were cephalosporins 74% aminoglycosides 36%, β lactams 14%, and fluoroquinolones 2%. The three parameters tested for adherence showed individual compliance of 92% for appropriate selection of antibiotics, 85% for the appropriate administration, and 56% for the appropriate duration of antibiotics, respectively.

Conclusion: The results highlight the challenges of disseminating evidence-based protocols systematically into routine clinical practice. Various measures are needed to improve appropriateness of prescriptions and adherence include the development of evidence-based guidelines in collaboration with surgeons, increased outcome-based research to document benefits of appropriate antibiotic use, continuing education to disseminate information to practitioners etc.

Keywords: Antibiotic prophylaxis, Guideline adherence, Surgery
Introduction

Prophylaxis is indicated for all procedures not classified as clean. As previously qualified, certain risk factors justify the use of prophylaxis for clean procedures as well. The following recommendations are provided for specific procedures. A recent quality standards report that further qualifies the strength of recommendations based on the quality of available supporting evidence is also useful.

Cutaneous and Superficial Soft Tissue Procedures - Prophylaxis is not indicated for cutaneous and superficial soft tissue procedures. For patients with two or more significant risk factors, prophylaxis is acceptable but not strongly indicated. Traumatic wounds require consideration of the status of the patient's tetanus vaccination. Although a single dose of antibiotics is acceptable, mechanical cleansing and adherence to guidelines for open management of wounds created more than 12 hours before treatment are the essential elements of prophylaxis.

Head and Neck Procedures - For procedures entailing entry into the oropharynx or esophagus, coverage of aerobic cocci is indicated. Prophylaxis has been shown to reduce the incidence of severe wound infection by approximately 50 percent [1,2]. Either penicillin or cephalosporin-based prophylaxis is effective. Cefazolin is commonly used. Prophylaxis is not indicated for dentoalveolar procedures, although prophylaxis is warranted in immunocompromised patients undergoing these procedures.

Neurosurgical Procedures - Studies evaluating the efficacy of antibiotic prophylaxis in neurosurgical procedures have shown variable results. The supportive data have been reviewed [2,3]. Nonetheless, prophylaxis is currently recommended for craniotomy and shunt procedures. Coverage targets *S. aureus* or *Staphylococcus epidermidis*. Various regimens have been assessed, ranging from combinations of cefazolin and gentamicin to single-agent therapy with cefazolin, vancomycin, piperacillin, and cloxacillin. No particular regimen has been clearly demonstrated to be superior. Until further data are available, therapy with cefazolin is considered appropriate.

General Thoracic Procedures - Prophylaxis is routinely used for nearly all thoracic procedures, despite the lack of available supportive evidence (most evidence is based on studies of pulmonary resection for lung cancer) [4,5]. In general, the strength of the recommendation is proportionate to the likelihood of encountering high numbers of microorganisms during the procedure. Pulmonary resection in cases of partial or complete obstruction of an airway is a procedure in which prophylaxis is clearly warranted. Likewise, prophylaxis is strongly recommended for procedures entailing entry into the esophagus. Although the range of microorganisms encountered in thoracic procedures is extensive, most are sensitive to cefazolin, which is the recommended agent.

Cardiac Procedures - Prophylaxis against *S. aureus* and *S. epidermidis* is indicated for patients undergoing cardiac procedures. Although the risk of infection is low, the morbidity of mediastinitis or a sternal wound infection is great. Numerous studies have evaluated antibiotic regimens based on penicillin, first-generation cephalosporins, second-generation cephalosporins, or vancomycin [6,7]. Although prophylaxis is efficacious, clear superiority of a particular regimen has not been demonstrated. In certain cases, results were institution-dependent, with exceptionally high rates of methicillin-resistant *S. aureus* or *S. epidermidis*. Such exceptions notwithstanding, cefazolin is an appropriate agent. Of particular relevance, cardiopulmonary bypass reduces the elimination of drugs, so additional intraoperative doses typically are not necessary.

The optimal duration of prophylaxis remains a debated topic, with many clinicians advocating prophylaxis for more than 24 hours, or until invasive lines and chest, tubes are removed. Most surgeons continue therapy for a minimum of 24 hours. Coverage until all lines and tubes are removed is not recommended or supported by data.

Gastrointestinal Tract Procedures - Prophylaxis is recommended for most gastrointestinal procedures. The number of organisms and the proportion of anaerobic organisms progressively increase along the gastrointestinal tract, so the recommendation depends on the segment of the gastrointestinal tract entered during the procedure. The intrinsic risk of infection associated with procedures entering the stomach, duodenum, and proximal small bowel is quite low and does not support a routine recommendation for prophylaxis. However, the predominance of clinical practice involves special circumstances that alter this recommendation. Any context associated with decreased gastric acidity is associated with a marked increase in the number of bacteria and the
Risk of wound infection. Therefore, previous use of antacids, histamine blockers, or a proton pump inhibitor qualifies the patient for prophylaxis. Prophylaxis is also indicated for procedures treating upper gastrointestinal bleeding. Stasis also leads to an increase in bacterial counts, so prophylaxis is warranted in procedures to correct the obstruction. In addition, the intrinsic risk of infection in patients with morbid obesity and advanced malignancy is sufficiently high to warrant prophylaxis in these cases. Although the local flora is altered in these patients, cefazolin provides adequate prophylaxis and is the recommended agent.

Colorectal procedures have a very high intrinsic risk of infection and warrant a strong recommendation for prophylaxis. Several studies have demonstrated efficacy, with rates of infection decreasing from over 50 percent to less than 9 percent [8,9,10]. Antibiotic spectrum is directed at gram-negative aerobes and anaerobic bacteria. Different strategies using parenterally or internally administered antibiotics are used, but all strategies are based on the use of mechanical bowel preparation with purgatives such as polyethylene glycol, mannitol, or magnesium citrate, given orally, and enemas.

Such pre-treatment decreases fecal bulk but does not decrease the concentration of bacteria in the stool. In fact, the risk of infection with mechanical preparation alone is still over 25 to 30 percent [9,10]. Therefore, additional prophylaxis is recommended. Options include either intraluminal (oral) prophylaxis directed at aerobic and anaerobic bacteria (given the day before operation) or the parenteral administration of similarly active antibiotics immediately before the operation [11]. In general, the addition of intraluminal antibiotics reduces the risk of infection to approximately 9 percent or less, similar to the risk associated with parenteral administration alone.

Trials comparing intraluminal preparation alone with intraluminal preparation plus parenteral administration have produced mixed results. The common practice among colorectal surgeons in the United States uses both intraluminal and parenteral prophylaxis, with the parenteral medication administered immediately before the operation [12].

Various intraluminal regimens appear to have similar efficacies. One recommended regimen consists of erythromycin base and neomycin given at 1 p.m., 2 p.m., and 11 p.m. (1 g of each drug per dose) the day before a procedure scheduled for 8 A.m. Times of administration are shifted according to the anticipated time of starting the procedure, with the first dose given 19 hours before surgery. Metronidazole can be substituted for erythromycin, and kanamycin (Kantrex) can be substituted for neomycin. If parenteral prophylaxis is desired, a second-generation cephalosporin with activity against anaerobic organisms is recommended. Cefotetan and cefoxitin are equally efficacious.

To summarize, recommendations for prophylaxis of colorectal procedures include the following: (i) mechanical cleansing beginning the day before surgery, typically continued until the effluent is clear (or until four to six hours before the start of the operation); (ii) neomycin and erythromycin base, 1 g of each medication orally at 1 p.m., 2 p.m. and 11 p.m. the day before surgery (or starting 19 hours before the anticipated starting time of the procedure), and (iii) intravenous administration of cefotetan or cefoxitin within 30 minutes of the time of incision.

Prophylaxis is also recommended for an appendectomy. Although the intrinsic risk of infection is low for uncomplicated appendicitis, the preoperative status of the patient’s appendix is typically not known. Cefotetan or cefoxitin are acceptable agents. Metronidazole combined with an aminoglycoside or a quinolone is also an acceptable regimen. For uncomplicated appendicitis, coverage need not be extended to the postoperative period. Complicated appendicitis (e.g., with accompanying perforation or gangrene) is an indication for antibiotic therapy, thereby rendering any consideration of prophylaxis irrelevant.

**Biliary Tract Procedures** - The recommendations for antibiotic prophylaxis for procedures of the biliary tract depends on the presence of specific risk factors. In general, prophylaxis for elective cholecystectomy (either open or laparoscopic) may be regarded as optional. Risk factors associated with an increased incidence of bacteria in bile and thus of increased risk for postoperative infection include age over 60 years, disease of the common duct, diagnosis of cholecystitis, presence of jaundice, and previous history of biliary tract surgery. Only one factor is necessary to establish the patient as high risk. In most cases of symptomatic cholelithiasis meeting high-risk criteria, cefazolin is an acceptable agent. Agents with theoretically superior antimicrobial activity have not been shown to produce a lower postoperative infection rate.
Obstetric and Gynecologic Procedures-
Prophylaxis is indicated for cesarean section and abdominal and vaginal hysterectomy. Numerous clinical trials have demonstrated a reduction in the risk of wound infection or endometritis by as much as 70 percent in patients undergoing cesarean section [13]. For cesarean section, the antibiotic is administered immediately after the cord is clamped to avoid exposing the new-born to antibiotics. Despite the theoretic need to cover gram-negative and anaerobic organisms, studies have not demonstrated a superior result with broad-spectrum antibiotics compared with cefazolin. Therefore, cefazolin is the recommended agent.

Urologic Procedures-
The range of potential urologic procedures and intrinsic risk of infection varies widely. In general, it is recommended to achieve preoperative sterilization of the urine if clinically feasible. For procedures entailing the creation of urinary conduits, recommendations are similar to those for procedures pertaining to the specific segment of the intestinal tract being used for the conduit. Procedures not requiring entry into the intestinal tract and performed in the context of sterile urine are regarded as clean procedures. It should be recognized, however, that prophylaxis for specific urologic procedures has not been fully evaluated.

Orthopedic Procedures-
Antibiotic prophylaxis is clearly recommended for certain orthopedic procedures. These include the insertion of a prosthetic joint, ankle fusion, revision of a prosthetic joint, reduction of hip fractures, reduction of high-energy closed fractures, and reduction of open fractures. Such procedures are associated with a risk of infection of 5 to 15 percent, reduced to less than 3 percent by the use of prophylactic antibiotics. S. aureus and S. epidermidis predominate in the wound or joint infections. Cefazolin provides adequate coverage. The additional use of aminoglycosides and extension of coverage beyond the operative period is common but lacks supportive evidence.

Noncardiac Vascular Procedures-
Available data support the recommendation for coverage of procedures using synthetic material, those requiring groin incisions and those affecting the aorta. Cefazolin is the recommended agent since most infections are caused by S. aureus or S. epidermidis. Prophylaxis is not recommended for patients undergoing carotid endarterectomy. Although two studies have demonstrated the efficacy of two postoperative doses of antibiotics [14,15], coverage for only the duration of the procedure is acceptable.

Breast and Hernia Procedures-
Various studies have clearly demonstrated a reduction in the risk of infection by administering prophylactic antibiotics to patients undergoing breast and hernia procedures, albeit the reduction of intrinsically low risk [16]. In general, prophylaxis is considered optional. For hernia repairs entailing the insertion of mesh, prophylaxis is considered desirable since the morbidity of infected mesh in the groin is substantial. However, no prospective trials demonstrate the effectiveness or necessity of this practice. Modified radical mastectomy and axillary node dissection also warrant prophylaxis, since wounds near or in the axilla have an intrinsic risk of infection. If prophylaxis is desired or indicated for any of these procedures, cefazolin is the recommended agent.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Criteria</th>
<th>Risk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean</td>
<td>Elective, not emergency, non-traumatic, primarily closed; no acute inflammation; no break-in technique; respiratory, gastrointestinal, biliary and genitourinary tracts not entered</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Clean-contaminated</td>
<td>The urgent or emergency case that is otherwise clean; the elective opening of respiratory, gastrointestinal, biliary or genitourinary tract with minimal spillage (e.g., appendectomy) not encountering infected urine or bile; minor technique break</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Contaminated</td>
<td>Nonpurulent inflammation; gross spillage from the gastrointestinal tract; entry into biliary or genitourinary tract in the presence of infected bile or urine; major break in technique; penetrating trauma &lt; 4 hours old; chronic open wounds to be grafted or covered</td>
<td>~ 20</td>
</tr>
<tr>
<td>Dirty</td>
<td>Purulent inflammation (e.g., abscess); preoperative perforation of respiratory, gastrointestinal, biliary or genitourinary tract; penetrating trauma &gt; 4 hours old</td>
<td>~ 40</td>
</tr>
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Laparoscopic and Thoracoscopic Procedures—Specific data supporting the recommendation of antibiotic prophylaxis for laparoscopic or thoracoscopic procedures are lacking. Therefore, pending the availability of new data, recommendations for the same procedure performed using the "open technique" should be followed.

Surgical antimicrobial prophylaxis reduces the risk of surgical site infection by around 50%. Guidelines recommend the type of antibiotic, the duration of treatment, the route of administration, and the dosage [16-19]. The choice of antibiotic should be based on the pathogens that are frequently associated with surgical site infection in a specific surgery.

A single administration is a rule for the vast majority of procedures. The treatment should never exceed 48 hours. Except for specific procedures as prostate surgery [19], the favorite route of administration is intravenous.

For cephalosporins, most guidelines recommend doubling the standard dose for obese patients even outside bariatric surgery.

Materials and Methods

Source of data: This is a retrospective cross-sectional study done over a period of 6 months from January 2019 to June 2019. Continuous 100 surgeries were selected. The study was conducted in the Department of Microbiology, in a tertiary care hospital. The demographic data regarding the surgeries were

Inclusion criteria: All the adult patients who went on elective surgery were included in the study.

Exclusion criteria
01. Emergency Surgeries
02. Neonates and pediatric surgeries
03. Presence of infection and/or antibiotics before surgery

Were excluded from the study

Methodology

The current study retrospectively reviewed 100 adult cases who underwent elective surgery in 2019 by picking them randomly from all the surgical departments. Details of the type of surgery, choice of antibiotic, a dose of antibiotic, time of administration in relation to incision time, duration of surgery, and administration of the intraoperative dose and continuation of antibiotics were recorded.

The hospital antimicrobial prophylaxis guideline was used as a benchmark for analyzing compliance and appropriateness of antibiotic prophylaxis in the 100 cases.

Follow up data included additionally administered doses of antimicrobial agents, the total duration of prophylaxis as well as signs and symptoms of surgical site infections.

If more than one drug was prescribed for a single procedure, all parameters for each drug were evaluated separately.

Appropriateness of preoperative antibiotic prophylaxis was assessed as per guidelines of CDC and hospital antibiotic policy. These guidelines provide evidence-based recommendations to the practitioners for the rational use of prophylactic antimicrobials.

Results

The medical charts were reviewed for demographic (age, sex) data, clinical data of patients were also collected.

Our records search showed that the mean age of the patients who underwent surgeries was 39 years and that 56% were females and 44% were males.

In the surgeries audited, cardiac was 38%, gastrointestinal surgeries were 26%, orthopedic and neurological cases were 10% each, and urology and OBG surgeries were 8% each.

![Fig-1: Different types of surgeries.](image-url)
Out of 100 surgeries that were audited only 4% were clean, 84% were clean-contaminated, 4% were contaminated and 8% were dirty.

The present study had more number of clean-contaminated surgeries than the clean in our hospital.

![Surgical wound classification as per the CDC.](image)

**Fig-2:** Surgical wound classification as per the CDC.

In the 100 cases, pre-surgical prophylaxis was given for 92% of cases and in 8% of cases, it was not followed.

![Compliance of Pre-surgical prophylaxis.](image)

**Fig-3:** Compliance of Pre-surgical prophylaxis.

The most commonly used antibiotics were cephalosporins 74%, aminoglycosides 36%, β lactams 14%, and fluoroquinolones 2%.

The three parameters tested for adherence showed individual compliance of 92% for appropriate selection of antibiotics, 85% for the appropriate administration, and 56% for the appropriate duration of antibiotics respectively.

Most of the surgeries the antibiotic was initiated at induction accounting to 41% of the total surgeries.

Out of 100 post-surgical case files, two antibiotics were most commonly used as pre-surgical prophylaxis accounting to 48%, closely followed by one antibiotic accounting to 44%. Pre-surgical prophylaxis was not used for 8% cases.

![Timing of antibiotic used before surgery.](image)

**Fig-5:** Timing of antibiotic used before surgery.

![Common groups of antibiotics used for surgical prophylaxis.](image)

**Fig-6:** Common groups of antibiotics used for surgical prophylaxis.

Cephalosporins were most commonly used accounting for 74 (74%) followed by β lactam+β lactam inhibitor combination accounting for 14(14%).

Among Cephalosporins, Cefuroxime was most commonly used accounting for 18. Furoquinolones were least commonly prescribed accounting for only one case (2%).

Among the combination of drugs Cefuroxime + Amikacin were most commonly used accounting to 30(62.50%) followed by Cephalosporins+Metronidazole and other combinations.
During the audit, it was observed that only 56% of Consultant surgeons have adhered to hospital antibiotic policy.

Discussion

The best timing for the surgical antimicrobial administration is based on a theoretical principle: the peak of antibiotic concentration at the surgical site should be reached at the time of incision. Thus, the timing depends on the pharmacokinetics of each antibiotic.

Guidelines provide divergent duration comprised between 30 and 60 min before incision. The administration of vancomycin and fluoroquinolones should be a starter within 120 minutes before surgical incision due to the prolonged infusion times required for these drugs [20].

However, the relation between the timing of the surgical antimicrobial prophylaxis and the incidence of surgical site infection remains unclear.

In a randomized clinical trial, Weber et al. administered 1.5 g of cefuroxime early (30–75 min before scheduled incision) in the anesthesia room or late in the operating room (0–30 min before scheduled incision) to 5,580 patients who were followed for a 30-day duration [21].

The antibiotic was given 42 min before incision in the early group and 16 min before incision in the late group. The rate of surgical site infection was 5.1%. It did not significantly differ in the early group and the late group. This finding was confirmed in each population: surgical division, wound class, immunosuppressive drugs, body mass index, diabetes, and age.

This randomized clinical trial is pragmatic, clear, and well-conducted. An impressive number of patients were included. The result, which does not support the “old theoretical model of pharmacokinetics”, is confirmed in each subgroup of patients, even those considered at high risk for surgical site infection. This study is a model for future studies: its pragmatic design makes it possible to clearly respond to a critical clinical question.

One of the limitations is probably the follow-up duration that was limited to 30 days, while surgical site infection in patients with prosthetic material should have been observed for 1 year. Can it really be believed that this limitation would change the main finding? Another limitation is that surgical antimicrobial prophylaxis represents one step of a series of measures aiming at preventing surgical site infection.

The WHO guidelines include 9 preoperative recommendations, 13 preoperative and/or intraoperative measures, and 3 postoperative measures [18]. Thus, one can suggest that it would be surprising that a few minutes in the administration of antibiotics play a major role in terms of outcome. In the present study, due to its design and the research constraints, the practices were probably optimal in the two groups.

In addition, the definition and surveillance of surgical site infections are not as consensual as they can first appear [22,23]. Finally, the authors tested the use of cefuroxime as surgical antimicrobial prophylaxis. The current study does not provide observations related to the results which could have been similar to other antibiotics.

In an observational study, the same group of authors suggested that the infection risk enhanced when surgical antimicrobial prophylaxis was administrated in the last 30 minutes before incision compared with a 31–60 minutes interval [24].

Another observational study concluded, at variance, that risk of surgical site infection was reduced when antimicrobial prophylaxis was infused in the last 30 minutes before incision [25]. Using an unadjusted model, a large study including 32,459 patients found higher rates of surgical site infection for timing more than 60 min prior to incision.

When the model was adjusted for patient, procedure, and antibiotic variables, no association was identified between antibiotic timing and surgical site infection [26].
In conclusion, a large scale randomized clinical trial and a well-conducted observational study showed that timing, if the deviation remains reasonable, i.e., between 30 and 60 min, is not critical for the prevention of surgical site infection. However, once again, in those studies, no major deviation, as prolonged delay or administration after surgical incision, was reported.

Weber et al. concluded that “even though the present study does not rule out a beneficial effect of early administration of surgical antimicrobial prophylaxis on the risk of surgical site infection, they do not support changing current recommendations to administer surgical antimicrobial prophylaxis during the 60 min before incision? [21].

He current study agrees with this pragmatic conclusion. The study results do not allow deviating from current WHO guidelines, which suggested randomized controlled trials to clarify the optimal timing of surgical antimicrobial prophylaxis.

However, the research agenda around the prevention of surgical site infection still require future investigations. In the intensive care unit, a continuous infusion of beta-lactams, after an initial bolus, is used to optimize the efficiency of antimicrobial treatments.

The time above the minimal inhibitory concentration of the causative pathogen is a critical determinant for its clearance. A meta-analysis showed a positive effect on the outcome of patients [27].

If this strategy was transferred to the operating room, at least for high-risk procedures, it would partly resolve the issue related to the timing of surgical antimicrobial prophylaxis administration.

Elsewhere, the long-term ecological effect of the surgical antimicrobial prophylaxis was never clearly assessed, which is a major bias in the era of increasing antimicrobial resistance.

The study of Weber et al. raises two comments. The first comment is the relevance of control quality studies. The timing between surgical antimicrobial prophylaxis and incision has been used as a surrogate for guideline adherence [28,29].

Depending on the study, a timing different from either 30 or 60 min was considered as an optimal practice. In a Dutch survey, the timing of the first dose was not in compliance with guidelines in 50% of cases [30], which can be penalizing in some circumstances. In addition, in routine practice, the timing between surgical antimicrobial prophylaxis and surgical incision appears difficult to control for all the operating room team. The present results raise questions about the interest of such quality criteria. This suggests that audit should preferentially focus on end-points that were confirmed in randomized clinical trials. The second comment is the value of randomized clinical trials.

The theoretical translation of concepts at the bedside did not often result in clinical success. Observational studies include inherent bias that makes their findings uncertain. International, national, and institutional organizations should support the use of randomized clinical trials in an attempt to improve the practices.

In conclusion, Weber et al. show that the timing of surgical antimicrobial prophylaxis does not affect the incidence of surgical site infection if its administration occurs in a reasonable range. One should keep in mind that surgical antimicrobial prophylaxis is a single element of a large bundle for the prevention of surgical site infection. This study also shows that randomized clinical trials remain mandatory in an attempt to confirm (or not) theoretical concepts.

The effectiveness of preoperative antibiotic prophylaxis is well established. Despite this, surveys have shown that optimal practice isn’t achieved in many hospitals [31].

In the present study, the majority (92%) of patients received antibiotic prophylaxis prior to surgery. This figure is comparable to those reported in previous studies from Turkey, Israel, and Greece. Among the study participants, 58% received antimicrobial combinations [32-33].

Potentially harmful aspects of such inappropriate antibiotic combinations include the emergence of resistant bacteria, super-infection, the risks of toxic and allergic reactions, and increased cost of therapy.

**Conclusion**

Surgical antimicrobial prophylaxis is the most common indication for antimicrobial use in hospitals. However, it is associated with high rates of inappropriate use Effective use of antimicrobials to prevent infection is essential to reduce risks associated with surgical procedures.
What does the study add to the existing knowledge?

Efforts need to be made to maximize the quality of surgical antimicrobial prophylaxis prescribing as it can have a significant role in optimizing surgical antimicrobial prophylaxis and reducing the burden of inappropriate antimicrobial use.

Author’s contributions

Dr. Shashikala N.: Study design

Dr. Manasa S.: Concept, manuscript preparation

Dr. Venkatesh Vikram H.C.: Statistical analysis

Reference

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