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FEATURE ARTICLE:

Inactive Ingredients used in Alcohol-Based Hand Sanitizers Marketed in the Nairobi Metropolitan Area

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The Pharmaceutical Society of Kenya (PSK) is a representative organization that was formed enabling Pharmacists' to employ their professional expertise in the care of patients.

Established in 1964, PSK has its roots in the Pharmaceutical Society of East Africa, which was registered in 1950. Since its formation, PSK continues to promote a common standard for professional conduct and code of ethics for its members, as well as advocate for the welfare of Pharmacists.

EDITORIAL

THE PHARMACIST IN THE COVID-19 ERA

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It has been more than 18 months since Covid-19 changed the world. Patients are still reporting different effects and symptoms including death. The pharmaceutical industry is working round the clock to provide the best vaccines while tackling supply chain issues. Timely vaccine access and coverage globally is a matter of life and death. The emergence of new variants reported in different continents has impacted on travel and tourism. Where does pharmaceutical care come in?

In the UK, Hospital pharmacists working in vaccination centres and hospital hubs have been given the "lead responsibility" for ensuring COVID-19 vaccines are safely handled and administered. Low and middle income countries are yet to acknowledge fully the role Pharmacists could play in managing this pandemic. Pharmacists globally require a common approach to vaccination to enhance the access and distribution of safe and effective vaccines.

In the US, the Federal Retail Pharmacy Program for COVID-19 Vaccination which is a collaboration between the federal government, states and territories, and several pharmacy networks are increasing access to COVID-19 vaccination in retail pharmacies. A clear program and schedule for vaccination will reduce infection rates particularly among vulnerable groups including the elderly and those with underlying conditions.

Clear patient education and counseling by Pharmacists especially the community Pharmacist is vital in developing countries where the majority of patients and clients seek first-line help prior to visiting hospital. This is part of primary health care. Pharmacists and the pharmacy workforce are frontline workers dispensing medication to patients and providing essential counseling and information. This contributes to high quality pharmacotherapy. Vaccination in retail pharmacies is an expanded role that is the future for a successful Universal Health Coverage program.

The inclusion of Pre-Service training in vaccination for Pharmacy programs is long overdue. These skills are required beyond the rich knowledge base of medicines and technologies that Pharmacists possess. A robust program that offers certification to licensed Pharmacists to administer vaccines will have a positive impact on the general population and ease the burden experienced in an overstretched national health system.

Continuous Professional Development among Pharmacists harnessing their IT skills will make them more adaptable to the ever-changing landscape brought about by the pandemic. Tele pharmacy opportunities are poorly explored but could create new business opportunities once the right infrastructure is in place. Self-care among Pharmacists and the pharmacy workforce by ensuring that everyone is vaccinated provides safer work spaces and better mental health while attending to patients and clients.

Use of Assay and *In-Vitro* Dissolution in a Tablet Manufacturing Case Study to Assess Batch to Batch Quality Consistency

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Abstract

Background: A pharmaceutical product is designed to deliver the drug substance to the target site in a required amount, desired rate, and with the same potency throughout their shelf-life. Batch to batch consistency is vital for ensuring product safety and efficacy. Any change that impacts a critical quality attribute may result in variable drug performance.

Objective: This study aimed to assess batch to batch consistency of metronidazole tablets manufactured by an indigenous company in Kenya.

Methodology: Assay and dissolution test results of twenty-four (24) batches of metronidazole tablets produced between the years of 2017 and 2020 were reviewed based on the company specifications for the product. X-bar control plots were used to determine out of control values and process variability. The distribution of the data set was characterized by both spread and central tendencies. In vitro dissolution profile analysis was performed on three (3) batches as per the official USP monograph using one of the batches as reference. Difference factor (f_1) and similarity factor (f_2) were calculated and evaluated.

Results: All batches complied with set specifications for assay and dissolution test. The assay ranged from 98.2% - 102.5% and dissolution values were 94.3% - 104.3%. Capability indices were >1; f_1 and f_2 values for the batches were (0, 1.27, 0.13) and (100, 87.14, 92.06), respectively.

Conclusion: The process capability indices and dissolution profiles verify the ability of the manufacturing process to repeatedly produce the established quality of metronidazole tablets with similar drug release pattern which ensure consistent batch to batch performance.

Keywords: *Assay, Batch, Consistency, Dissolution, Quality.*

Introduction

The US Food and Drug Administration (FDA) defines a quality drug as a contamination free product that can consistently provide therapeutic benefits to the user, as specified by the label claim [1]. The pharmaceutical product is expected to deliver the drug to the patient in a required amount, required rate, and with the same potency throughout their shelf-life. The quality of a drug is strictly controlled to ensure its fitness for use by setting of quality standards such as disintegration, assay and dissolution [2, 3]. Dissolution testing is an *in vitro* laboratory performance test that assesses how efficiently a drug is released from its dosage form while the assay determines the overall potency of the batch.

Maintaining batch to batch consistency in pharmaceutical production is essential for ensuring quality and efficacy of the product [4-6]. Reproducible batch operations are key to consistency in pharmaceutical processes and this is achieved through stringent adherence to Good Manufacturing Practice (GMP) regulations promulgated by Drug Regulatory Authorities (DRAs) [7-11]. A sound understanding of critical material attributes and process parameters is essential in identifying probable sources of variability. Further, DRAs require that any change to a registered pharmaceutical product should be made according to provision stipulated in variation guidelines to ensure that quality and efficacy of the product are not compromised [12, 13].

To secure quality of products consistently, control of raw materials, critical process parameters and process consistency are necessary. It has been recognized that variability in material properties of both the drug substance and excipients can have profound effects on the final product performance [14, 15]. Dissolution test is an important parameter to characterize drug product performance since release of the drug from a solid dosage form often impacts its rate and extent of absorption. Factors that affect dissolution of a drug product include the intrinsic properties of the drug substance (for example; solubility, particle size, polymorphs), the formulation composition and the manufacturing process [16]. This was demonstrated by tolbutamide where tablets with slower disintegration showed a marked decrease in plasma levels. In regard to phenytoin, increased toxicities were observed when calcium sulphate was substituted with lactose [17].

The dissolution test enables detection of influence of key manufacturing factors including excipients and provides better control of the production process thus assuring

consistent batch to batch quality of the product. Dissolution is an important *in vitro* test of tablet quality because of its correlation with drug bioavailability [18]. The test is used during approval of generic dosage forms where *in vitro* bioequivalence studies can be avoided especially for highly soluble drug substances [19]. Comparative dissolution profiles are evaluated statistically by use of difference factor (f_1) and similarity factor (f_2) [20-23]. The f_1 compares results at the same time points, whereas f_2 compares the overall profiles. The f_1 value should be between 0 and 15, whereas f_2 should be between 50 and 100 for two dissolution profiles to be considered similar.

Batch to batch consistency of pharmaceutical products is desirable to ensure bioavailability, safety and efficacy. However, variation in batches of the same product may arise in situations where production materials are inappropriately controlled, quality by design (QbD) principles are not applied in product development to identify critical quality parameters and post approval changes in manufacture of a product are inadequately evaluated [24, 25]. Additionally, there are instances when raw materials are sourced from brokers without manufacturer's identity and furthermore, changes may be made to improve capacity without adequate scale-up procedure. Change of manufacturing site, batch size variation and deviation from manufacturing procedure may impact quality of the product if the changes are inappropriately controlled [13]. It is noteworthy that during pharmaceutical product approval, regulatory authorities require comparison of one batch of generic product with innovator product and batch to batch differences within a product are not assessed. As such, majority of in vitro dissolution profile studies do not focus on inter batch variations.

In this study, batch to batch consistency of metronidazole tablets 400 mg manufactured by Elys Chemical Industries Ltd. of Kenya was assessed by evaluation of assay and dissolution test results of twenty-four (24) batches that were produced between the years of 2017 and 2020. In addition, batch manufacturing records were reviewed to identify any critical deviations or changes during production process that could possibly impact performance of the finished product. Metronidazole tablets contain metronidazole; a nitroimidazole derivative that is used for treatment of bacterial and protozoal infections. Metronidazole is the drug of choice for symptomatic intestinal and extraintestinal amebiasis, a leading cause of severe diarrhoea globally and prevalent in developing countries due to inadequate public sanitation [26]. It is also used to treat infections caused by *Trichomonas vaginalis, Giardia lamblia, Gardnerella vaginosis* and anaerobic infections such as intra-abdominal abscesses, gynaecological infections of the pelvic organs, soft tissue infections and osteomyelitis. Metronidazole tablets are designed for immediate release of the drug substance and manufactured by wet granulation method. The granulation process enables particle enlargement by agglomeration to generate the desired granule properties intended for the specific purpose and significantly impacts dissolution characteristics of solid dosage forms [27-29].

Methodology

Data collection and review

Twenty-four batches of metronidazole 400 mg tablets manufactured by Elys Chemical Industries Ltd. between January 2017 and October 2020 were evaluated. Metronidazole tablets contain the active pharmaceutical ingredient (API) and four (4) excipients; lactose, maize starch, pre-gelatinized starch and potassium sorbate. A comprehensive quality review of the finished product dissolution test results, dissolution profiles, assay and production process elements that impact dissolution was conducted.

Batch manufacturing record review

Batch Manufacturing Records (BMRs) were examined for compliance of pharmaceutical ingredients in the twenty-four (24) batches with the master formula record. The BMR entries for critical granulation process variables including quantity of binder fluid, impeller speed, chopper speed and granulation time were reviewed for conformity with pre-set values. The BMRs were examined to identify any critical deviations or changes during production process that could possibly impact performance of the finished product.

Assay, dissolution test and dissolution profiles

Manufacturer certificates of analysis (COAs) for the raw materials used in these batches were verified by analyzing the information on tests performed, test results and manufacturing site for conformance to the corresponding monograph of the material. Quality control test report for each material was reviewed for compliance with the relevant standard specification. Dissolution and assay test results for the manufactured tablets were obtained from quality control database and these were evaluated for compliance with existing quality standard for the two (2) parameters. Content of metronidazole per tablet was determined by non-aqueous titration method outlined in the individual drug monograph of the British Pharmacopoeia. Dissolution testing was carried out according to the official USP monograph for metronidazole tablets. The dissolution medium was 0.1 M Hydrochloric acid and sample solution was withdrawn after 60 minutes.

The samples were analyzed by UV-Visible spectrophotometric method in comparison with metronidazole working standard. The absorbance of both the standard and sample solution was measured at 278 nm using UV-Visible spectrophotometer (Shimadzu UV-1800) with the dissolution medium as the blank. Comparative dissolution profiles were performed following change of granulator machine to determine pre and post change similarity in drug release from the solid dosage form. Three batches were assessed, one having been manufactured prior to the change and this was used as a reference. Twelve tablets were tested for each batch. Sampling was performed at timed intervals of 10, 15, 30, 45 and 60 minutes.

Data treatment and analysis

The assay and dissolution test results for the twenty-four (24) batches were subjected to statistical analysis with Microsoft® Excel 2019 functions. The mean ($\bar{x}$) and standard deviation (σ) for each attribute were calculated and plotted to generate X-bar chart for monitoring process mean and distribution of the values within the specification limits. The upper and lower control limits (UCL and LCL) were computed according to Equations 1 and 2. The upper and lower process capability indices; C_{pku} and C_{pkl} were calculated from Equations 3 and 4. The lower value between C_{pku} and C_{pkl} was taken as process capability index (C_{pk}). Difference factor (f_1) and similarity factor (f_2) for the dissolution profiles were calculated using all five points for each batch as per Equations 5 and 6.

$$UCL = \bar{x} + 3\sigma \text{ (Equation 1)}$$

$$LCL = \bar{x} - 3\sigma \text{ (Equation 2)}$$

$$C_{pku} = \frac{[USL - \bar{x}]}{3\sigma} \text{ (Equation 3)}$$

$$C_{pku} = \frac{[\bar{x} - LSL]}{3\sigma} \text{ (Equation 4)}$$

$$f_1 = \{[\Sigma_{t=1}^{n} (R_t - T_t)] [\Sigma_{t=1}^{n} R_t]\} \times 100 \text{ (Equation 5)}$$

$$f_2 = 50 \times Log\{[1 + (1/n) \Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\} \text{(Equation 6)}$$

Where n is the total number of the time points selected.

R_t is the % API dissolved at each of the selected 'n' time points for Reference product.
T_t is the % API dissolved at each of the selected 'n' time points for test product.

Results

Batch manufacturing record review

The active pharmaceutical ingredient and excipients that were used in production of the twenty-four (24) batches of metronidazole tablets are presented in Table 1. These materials were sourced from prequalified vendors. The number of batches of each ingredient that were utilized in production of the tablets ranged from four (4) to thirteen (13). The difference in the number of batches is mainly attributed to the quantity of the ingredient that is used per tablet. Quality control analysis results for all ingredients conformed to the preset specification standards for identity, physical parameters, loss on drying (LoD), chemical tests, assay, related substances, microbial contamination and other relevant tests as tabulated under the compliance attributes column for each material. Table 2 presents dissolution impacting process parameters that were evaluated. All BMR entries for critical granulation process variables; quantity of binder fluid, impeller speed, chopper speed, granulation time, drying time, drying temperature (inlet and outlet), moisture content of granules and sifting mesh size were consistent with the master record of the finished product and BMR. No deviations or out of specification data entries were observed in the BMRs. All batches were processed in conformance to the control limits which were specified in the batch manufacturing record.

Table 1. Analysis results of pharmaceutical ingredients used in metronidazole tablets.

Ingredient	Number of Batches	Compliance Attributes
Metronidazole (API)	13	Description, solubility, identification, visible impurities, related substances, appearance of solution, LoD, sulphated ash, assay.
Maize starch	7	Description, solubility, identification, pH, LoD, microbial contamination, sulphated ash, oxidizable substances.
Lactose	5	Description, solubility, visible impurities, identification, appearance of solution, acidity/ alkalinity, specific optical rotation, absorbance, heavy metals, water, sulphated ash, microbial contamination.
Pregelatinized starch	8	Description, solubility, identification, pH, oxidizing substances, iron, foreign matter, LoD, sulphated ash, microbial contamination.
Potassium sorbate	4	Description, solubility, visible impurities, identification, appearance of solution, acidity/ alkalinity, aldehydes, heavy metals, LoD, Assay.

Table 2. Dissolution impacting process parameters.

Stage	Parameter	Specification
Dry blending	Impeller speed Chopper speed Mixing time	75 rpm 1500 rpm 5 minutes
Wet blending	Impeller speed Chopper speed	75 rpm 1500 rpm
Stage 1	Mixing time Granulation fluid	11 minutes 40 L
Stage 2	Impeller speed Chopper speed Impeller load Mixing time	100 rpm 2000 rpm 42-44 amperes 6 minutes
Granule discharge	Impeller speed Chopper speed	3 rpm 600 rpm
Drying	Inlet temperature Outlet temperature	53°C 35°C
Semi-drying & milling	Drying time Mesh size	1st cycle 15 minutes 2nd cycle 30 minutes # 6
Final drying	Inlet temperature Outlet temperature LoD	55°C 32°C 2-4%
Sifting	Mesh size	# 40

Assay and dissolution test

A summary of assay and dissolution test results for the twenty-four (24) batches of metronidazole tablets (range, mean, standard deviation, control limits and capability indices) is presented in Table 3. The assay results ranged from 98.2% - 102.5% and dissolution values were 94.3% - 104.3%. All batches complied with the set specifications of assay (95.0% - 105.0%) and dissolution (85%(Q)). The low standard deviation observed indicates that most of the data points were clustered close to the mean. Statistical analysis of the results using X-bar control chart to monitor process variability for the two parameters is displayed in Figures 1 and 2. The upper and lower control limits of assay test dataset were 103.98% and 97.11%, respectively. The lower control limit of dissolution test; a one-sided specification

attribute was 92.87%. All test results for the two attributes were within the established control limits. From the control charts, there was no peculiar trend observed in the data values. The assay and dissolution test results were adequately centered between the specification limits. The process capability index (C_{pk}) for assay and dissolution tests was 1.3 and 2.29, respectively.

Dissolution profiles

The comparative dissolution profiles for three (3) batches of metronidazole tablets; A, B and C are graphically presented in Figure 3. For the three (3) batches, 100% of the labelled amount of API was dissolved in the media within 10 minutes and the average dissolution at each time point was as indicated. The f_1 and f_2 factors for the reference batch (A) were presumed to be 0 and 100, respectively. The f_1 and f_2 values for B were 1.27 and 87.14, respectively. The calculated f_1 and f_2 values for the three (3) batches are shown in Table 4. In all cases, f_1 was less than 15 whereas f_2 lay between 50 and 100, hence the three dissolution profiles are considered to be similar.

Table 3: Assay and dissolution quality data (n=24 batches)

Parameter	Dissolution (%)	Assay (%)
Range	94.3-104.3	98.2-102.5
$\bar{x}$	98.95	100.54
σ	2.03	1.14
3σ	6.08	3.46
UCL	NA	103.98
LCL	92.87	97.11
USL	NA	105
LSL	85	95
USL-$\bar{x}$	NA	4.46
$\bar{x}$-LSL	13.95	5.54
Cpku	NA	1.30
Cpkl	2.29	1.61
Cpk	2.29	1.30

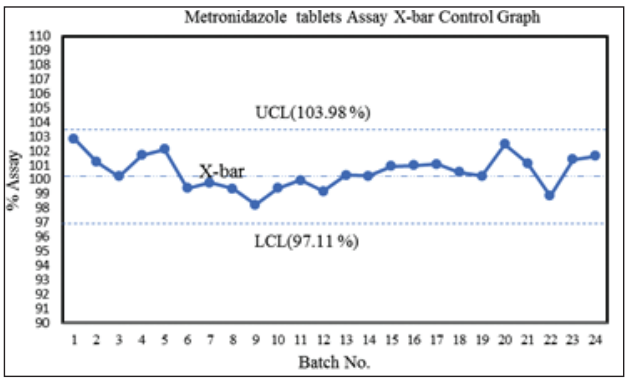

Figure 1. X-bar chart; Assay results (n=24 batches)

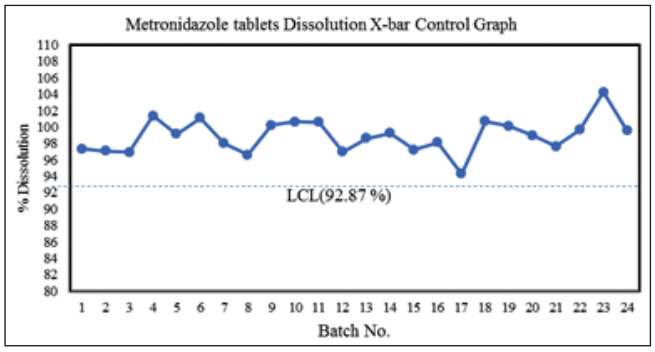

Figure 2. X-bar chart; Dissolution test results (n=24 batches)

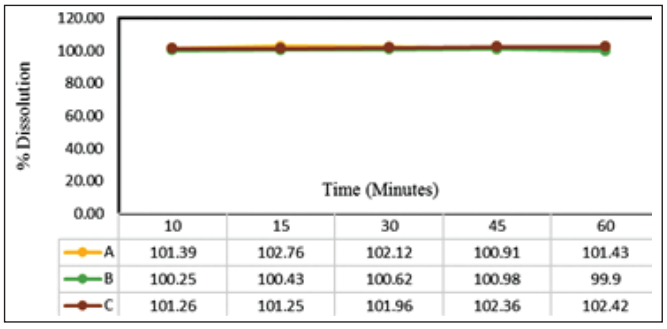

Figure 3. Comparative dissolution profiles (n= 3 batches)

Table 4. Calculated difference and similarity factors of three test batches (A,B,C)

Batch number	f_1	f_2
A (reference batch)	0	100
B	1.27	87.14
C	0.13	92.06

Discussion

A fundamental evidence of GMP compliance is consistent product quality. In this study, batch to batch consistency of metronidazole tablets produced between the years of 2017 and 2020 at one manufacturing site in Kenya (Elys Chemical Industries Ltd.) was assessed by review and evaluation of two critical quality attributes of the product; assay and dissolution. Finished product quality control test results for the two attributes were within predetermined specifications for the twenty-four (24) batches that were manufactured during this period. X-bar control charts for these attributes demonstrated that product variability is under control and the process capability indices verified consistency of the manufacturing process. Dissolution profiles for three of the tested batches (A,B,C) were similar at every dissolution sample time point and exhibited overall profile similarity.

The results show that the established critical quality attributes of metronidazole tablets were consistently produced. The capability indices (C_{pk}) for dissolution (2.29) and assay (1.3) denote that all test results were within specification limits and appropriately distributed. The manufacturing process is therefore considered to be capable of consistently producing outputs that are within specifications. The dissolution difference and similarity factors for the three batches; f_1 (0, 0.13, 1.27) and f_2 (100, 87.14, 92.06) imply that these profiles are similar, and signify batch to batch consistency.

Consistency in pharmaceutical production is mostly achieved through strict adherence to GMP, understanding of critical material attributes during product development, identification of critical process parameters and a robust change management system for ensuring that all changes impacting critical quality attributes are adequately controlled. Implementation of a sufficient validation program and adequate skilled production personnel may have contributed to consistency in the output. The manufacturer had sufficient control on raw materials with adequate control of critical process parameters impacting

dissolution. It is also probable that this manufacturer has inculcated GMP culture in production processes with an effective change control standard procedure for ensuring that any change impacting quality is adequately defined, reviewed and approved before implementation. This was demonstrated by the performance of comparative dissolution profile after the change of granulator. In addition, metronidazole drug substance is a class I drug in biopharmaceutics classification system (BCS) (highly soluble and highly permeable) [30], consequently, batch to batch variation is improbable in dissolution test results. However, it should be noted that excipients affect pharmaceutical performance due to their influence on release of the drug substance.

The risk associated with changes in production materials without adequate assessment may be catastrophic. For example, in the case of phenytoin, increased toxicities were observed when the manufacturer substituted calcium sulphate with lactose [17]. A spate of anticonvulsant intoxication occurred in 87% of epileptic patients. This illustrates the necessity of adequate material control and application of QbD principles in product development to ensure batch to batch consistency and avoid precarious consequences. Another study showed that dissolution rates of three batches of an immediate release analgesic drug from one manufacturer varied greatly (95.9%, 84.9% and 32.3%) [31]. Changes in formulation, production process and production capacity such as batch reduction or scale up require strict GMP compliance to ensure that there is no variability in pre- and post-change batches.

A number of pharmaceutical manufacturers in developing countries where production of raw materials is limited often encounter difficulties in Supplier/Vendor evaluation. These materials are usually purchased from brokers, in small quantities and frequently lack vital information such as physical characteristics and manufacturing site. This scenario may lead to variability in critical material attributes that impact drug product performance. The change may not be detected since performance tests such as comparative dissolution profile determination are not routinely conducted for batch release. Pool qualification of raw materials through partnerships with other manufacturers in the industry is an approach that could be considered to leverage on material quantity in order to facilitate direct purchase from manufacturers. Additionally, the manufacturers should perform regular product quality reviews for all approved products to demonstrate consistency in product quality. Furthermore, stringent GMP compliance audits by DRAs are vital for ensuring adequate material control and adherence to guidelines on variations to an approved pharmaceutical product [13].

The main limitation of this study is that evaluation of only one product is presented. A comprehensive analysis of one product from each of the dosage forms produced at this site may be a more accurate presentation of quality consistency of pharmaceutical products that are manufactured.

Conclusion

A study of assay and *in vitro* dissolution of metronidazole tablets 400 mg produced by an indigenous company in Kenya was carried out to assess batch to batch consistency. Twenty-four batches of metronidazole tablets that were manufactured from 2017 to 2020 were evaluated. All the batches complied with established specifications for assay and dissolution test and also exhibited similar drug release profiles. The control charts demonstrated that product variability was under control. Process capability indices verified consistency and robustness of the manufacturing process. This study shows that the established critical quality attributes of metronidazole tablets were repeatedly attained which assures consistent batch to batch performance.

Conflict of interest

No conflict of interest was declared by the authors.

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Antimicrobial Use Practices in a Kenyan Health Care Facility: A Point Prevalence Survey

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Abstract

Background: AAntimicrobials are indispensable in the practice of medicine. Their misuse is a great force behind the rapid growth of resistance, risk of serious drug reactions, poor treatment outcomes and waste of resources. Antimicrobial resistance is a grave and growing public health threat today. Advocating and promoting rational use of antimicrobial agents through antimicrobial stewardship programs is pivotal in curbing increasing growth of resistance.

Objective: To establish antimicrobial use practices in a Kenyan health care facility.

Methodology: A Point Prevalence Survey was conducted in all wards of Mbagathi Hospital, in Nairobi City County. Universal sampling was employed, whereby all patients who met the inclusion criteria were included in the study as stipulated in the global point prevalence survey protocol of 2018. Patient demographic and clinical data were extracted from the patient files, treatment sheets, and laboratory culture and sensitivity reports. All raw data collected was entered into EPI info version 7 and a database created. Descriptive and linear regression data analysis was conducted.

Results: A total of 185 patient records were sampled of whom 146 (78.9%) received at least one antimicrobial. Overall, 363 antimicrobials were prescribed during admission. Each participant was prescribed two antimicrobials on average. The most important risk factors for number of antimicrobials used were HIV status, prior hospitalization in the last 90 days, catheterization and nutritional status. Antibiotics formed the biggest proportion of antimicrobials prescribed in Mbagathi Hospital (n=294, 81%) followed by antivirals (n=48, 13%). Most prescribed antimicrobial was ceftriaxone at 86 (23.7%) and the commonest indication was pneumonia with a prevalence of 78 (33%). Culture and sensitivity tests were only ordered in (n=7, 3.8%) of the cases.

Conclusion: PPoor prescribing practices were observed. The prevalence of antimicrobial use was above the World Health Organization reference value of 30% or less. Ceftriaxone was used to a great extent. Empiric prescribing was mainly the practice as culture and sensitivity testing were not routinely done. The hospital medicines and therapeutics committee should set up an antimicrobial stewardship committee to help in judicious antimicrobial use.

Keywords: *Antimicrobial, Antimicrobial resistance, Point prevalence survey.*

Introduction

Majority of medicines are sub-optimally prescribed, dispensed or marketed especially in the developing world where drug regulatory mechanisms are in their infancy stages of development or not available [1]. Antimicrobial resistance (AMR) is a serious global concern and is considered one of the greatest dangers to human existence. According to World Health Organization (WHO) AMR Global Resistance Report on Surveillance, April 2014, AMR is a grave phenomenon in many parts of the world [3]. According to AMR Global Resistance Report on Surveillance 2014, there is high prevalence of resistance to third generation cephalosporins by *Esherichia coli* and *Klebsiella pneumonia*. This therefore means that severe infections by these bacteria have to rely on carbapenems which are reserved as the last resort. Carbapenems are costly, and may be unavailable in poor settings. Of very worrying concern is that up to 54% of *Klebsiella pneumoniae* are resistant to carbapenems [2].

The world is unable to keep abreast with increasing AMR to current treatments. Furthermore, the number of new classes of antibiotics has also dramatically declined over the past four decades. This means that the prospects of getting into a post antibiotic period are real [4]. Antimicrobial resistance is not only a concern with antibiotics but also poses a danger in the successful prevention and care of a continuously growing variety of viral, fungal and parasitic infections [5]. Overusing and misusing antimicrobials increases AMR rates [6], and hence there is crucial requirement to control as well as monitor the use of the existing antimicrobials [7]. Multidimensional initiatives such as prohibiting over the counter sale of antimicrobials, delayed prescribing strategies, develop and implement treatment guidelines, and institute Antimicrobial Stewardship Programmes (ASPs) [1] may be helpful. Other activities include regular clinical audits, use of valid rapid diagnostic tests, pragmatic studies on complications and clinical outcomes plus improvement of

communication proficiency with patients [1, 5]. Such initiatives have been implemented across continents including some African countries like Botswana [8]. The aim of this study was therefore to establish antimicrobial use practices among admitted patients in a Kenyan health care facility.

Methodology

Study design

The study was a l Point Prevalence Survey (PPS). Universal sampling was done therefore a minimum sample size was not computed. All patients admitted before 8 am on the day of the survey were sampled. According to the Global Point Prevalence Protocol of 2018, in hospitals with <500 bed capacity, all patients who meet the inclusion criteria should be included in the study [9]. Data was collected in a single day for each ward.

Study site and population

The study was conducted at Mbagathi Hospital, Nairobi City County-Kenya. It is a 200 bed capacity facility with several wards. The Hospital serves an urban catchment area with over 3.1 million Kenyans. Of these 22% live below the poverty line. The hospital admits over 2000 paediatric patients yearly and over 5000 adults.

All inpatients admitted during the survey in the internal medicine, paediatric, surgical, maternity, and post natal and the new born unit were included in the study The study was conducted between March and April, 2019. Participants with missing or incomplete records or admitted for same day procedures were excluded.

Data Collection instruments and procedures

The Global Point Prevalence Survey (G-PPS) data collection forms were adapted [10]. A ward data collection form was used to record information on the name of the ward and type of patients admitted therein. The data collection form was used to record the patient's bio-data, medicines prescribed, duration and all accompanying information on any antimicrobials prescribed. This study involved extraction of data from the patient files, treatment sheets and laboratory culture and sensitivity reports.

Quality Assurance

The ward data collection and the patient data collection tools were pre-tested before the study commenced. Research assistants constituted two registered pharmacists and one registered medical officer working at the facility at the time of the study.

Data Management and Analysis

Patient information was coded and no patient identifiers were used. Data collection instruments were kept under key and lock and the computer files password protected. Regular backup of the database was done to guarantee data integrity. All raw data was entered into Epi info version 7 software and later exported to STATA version 14.2 for analysis. Bivariable linear regression analysis was done to identify risk factors for prescribing multiple antimicrobials. The level of significance was set at 0.05.

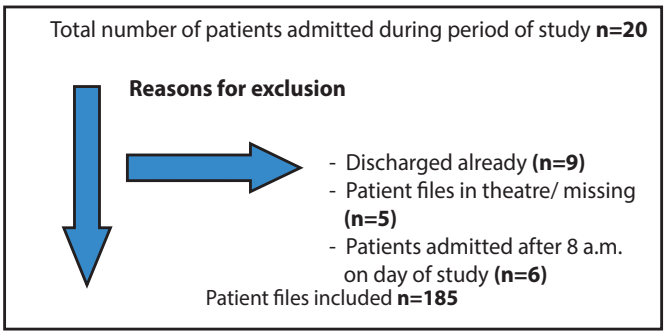
Ethical considerations

Approval to carry out this study was sought from the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee in February 2019 (Ref.no.KNH-ERC/RR/48). To implement the study, permission was sought from the Mbagathi Hospital research committee in March 2019, (NO.MDH/RS/1/VOL.1).

Results

Participants Recruitment and Enrollment

Data was collected over three weeks. A total of 205 patients were screened for eligibility of which 185 met the criteria. Twenty patients were excluded for various reasons as presented in Figure 1.



Figre 1. Consort diagram showing how 185 participants were included in the study.

Socio-demographic Characteristics of Study Participants

Table 1 is a summary of the baseline characteristics of the 185 participants who met the inclusion criteria. Majority of the participants were above 18 years (n=108, 58%) and the fewest were children between 1 and 17 years (n=19, 10.3%). More than half of the participants were female (n=110, 59%). A large proportion of patients came from medical ward (n=51, 28%) followed by paediatrics (n=48, 26%). The least number of patients were from the surgical wards (n=21, 11%).

Table1. Social Demographic Characteristics of survey Participants.

Characteristic		n (%)
Age in years	Adult (≥ 18 Years)	108 (58.4)
	Child (≥ 1 and ≤ 17 Years)	28 (15.1)
	Infant (≥ 1 and ≤ 11 Months)	19 (10.3)
	Neonate (≤ 28 Days)	30 (16.2)
Gender	Female	110 (59.5)
	Male	75(40.5)
Ward type	Maternity	40 (21.6)
	Medical	51 (27.6)
	Nursery	25 (13.5)
	Paediatrics	48 (26.0)
	Surgery	21 (11.4)

Medical characteristics

There were significant correlations between the ward type and most of the medical characteristics observed (Table 2).

Out of the 185 patients sampled, 79 (42.7%) had been referred from other facilities and the bulk 33 (64.7%) were in the medical ward. A total of 145 patients were catheterized at some point during their hospital stay. This constituted 78% of total patients surveyed. Almost half of the patients) n=91, 49%) were malnourished and 38 (74.5%) were in the medical ward. Thirty-four (18.4%) were HIV positive of which 29 (56.9%) were admitted in the medical ward. Most of the patients on TB treatment (27, .52.9%) were also from the medical ward.

Table 2. Medical characteristics of study participants.

Variable	Maternity	Medical	Nursery	Paediatric	Surgery	p-value
Referred from another facility	3 (7.5)	33 (64.7)	3 (12)	26 (54.2)	14 (66.7)	**<0.001**
Catheterized	15 (37.5)	46 (90.2)	23 (92.0)	46 (95.8)	15 (71.43)	**<0.001**
Intubated	0 (0.0)	4 (7.8)	2 (8.0)	3 (6.3)	2 (9.5)	**0.473**
Malaria test done	1 (2.5)	13 (25.5)	0 (0.0)	38 (79.2)	0 (0.0)	**<0.001**
Malnourished	2 (5.0)	3 (74.5)	18 (72.0)	28 (58.3)	5 (23.8)	**<0.001**
HIV +ve	2 (5.0)	29 (56.9)	0 (0.0)	2 (4.17)	1 (4.8)	**<0.001**
On TB treatment	0 (0.0)	27 (52.9)	0 (0.0)	3 (6.25)	1 (4.76)	**<0.001**

History of Antimicrobial Use

Nearly 1 out of 4 patients had used an antimicrobial before admission. Prior use was particularly high amongst paediatric patients. In this sub-population, slightly over 80% had been treated with an antimicrobial before admission. Amoxicillin was the commonly used antimicrobial.

Antimicrobials prescribed during admission at the facility

Of the 185 patient records sampled, 146 (78.9%) had one or more antimicrobial agents. A total of 363 antimicrobials were prescribed during admission. There were statistically significant differences in the patterns of prior antimicrobial use. Paediatric ward had the highest prevalence.

Types of antimicrobials prescribed

Antibiotics formed the biggest proportion of antimicrobials prescribed during admission in Mbagathi Hospital, n=294(81%). Antivirals n=48(13%) followed and the least prescribed were antimalarials and antifungals at 3% each. The most commonly used antimicrobial for surgical prophylaxis was ceftriaxone and co-trimoxazole was used widely for medical prophylaxis.

Distribution of number of antimicrobials prescribed per patient

The number of antimicrobials prescribed per patient ranged from 1 to 8. On average 2 antimicrobials were prescribed per patient, though extremes of up to 6 and 8 antimicrobials prescribed to a single patient were noted. Figure 2 shows the overall frequency distribution of the number of antimicrobials per patient.

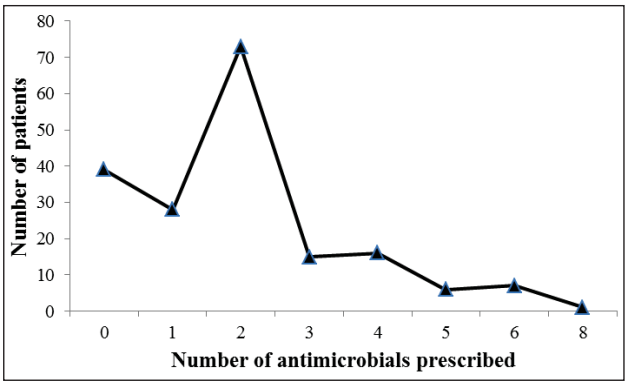


Figure 2. Frequency of individual antimicrobials prescribed at Mbagathi Hospital.

The most commonly prescribed antimicrobial was ceftriaxone at 23.7% (n=86) followed by gentamicin (10.2%), metronidazole (9.4%), RHZE (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol) (9.1%) and co-trimoxazole (8.5%). The top five antimicrobials were all antibiotics. Some low frequency antimicrobials included dolutegravir, amphotericin-B, norfloxacin and vancomycin. This is illustrated in Table 3.

Table 3. Frequency of individual antimicrobials prescribed at Mbagathi Hospital.

Antimicrobial	n (%)	Antimicrobial	n (%)
Ceftriaxone	86 (23.7)	Clindamycin	3 (0.8)
Gentamicin	37 (10.2)	NVP*	3 (0.8)
IV Metronidazole	34 (9.4)	Oral Metronidazole	3 (0.8)
RHZE*	33 (9.1)	Benzathine penicillin	2 (0.6)
Co-trimoxazole	31 (8.5)	Clarithromycin	2 (0.6)
TDF/3TC/EFV*	31 (8.5)	Cefuroxime	2 (0.6)
Benzyl penicillin	28 (7.7)	Meropenem	2 (0.6)
Acyclovir	10 (2.8)	Nystatin	2 (0.6)
Ceftazidime	8 (2.2)	DTG*	1 (0.3)
Fluconazole	8 (2.2)	TDF/3TC*	1 (0.3)
Amikacin	7 (1.9)	Amphoterin B	1 (0.3)
AL*	6 (1.7)	AZT*	1 (0.3)
Flucloxacillin	6 (1.7)	ABC/3TC/AZT*	1 (0.3)
Erythromycin	5 (1.4)	Norfloxacin	1 (0.3)
Artesunate	4 (1.1)	Vancomycin	1 (0.3)
Amoxicillin	3 (0.8)		

RHZE*- Rifampicin Isoniazid Pyrazinamide Ethambutol, TDF/3TC/EFV*-Tenofovir Lamivudine Efavirenz, AL* Artemether Lumefantrine, NVP* Nevirapine, DTG* Dolutegravir, TDF/3TC* Tenofovir Lamivudine, AZT* Zidovudine, ABC/3TC/AZT* Abacavir Lamivudine Zidovudine.

Duration, Frequency and Route of antimicrobial use

Most of the antimicrobials were prescribed once a day (46%), followed by twice daily at 29%. The most common route of administration was intravenous at 59%. Oral route accounted for 41% of prescribed antimicrobials. Most of the antimicrobials were prescribed over a period of 4-7 days (26.4%). Several antimicrobials accounting for 53.2% had no duration of use on the treatment sheet. This meant that the

nurses administered them daily until discharge. A stop review date was available for 60.9% (221) of the antimicrobials prescribed but 39.1% (142) had no documentation on when to stop or review treatment. Almost half of the patients admitted (45.5%) missed at least one dose during their hospitalization with some missing up to 30 doses.

Indications for antimicrobial use

Pneumonia was the most common indication for antimicrobial use across the wards (n=78) followed by, prophylaxis for obstetric gynecology surgical cases (n=32). Tuberculosis was third, (n=31), general medical prophylaxis fourth n=28, and sepsis n=21. This is illustrated below (Figure 3).

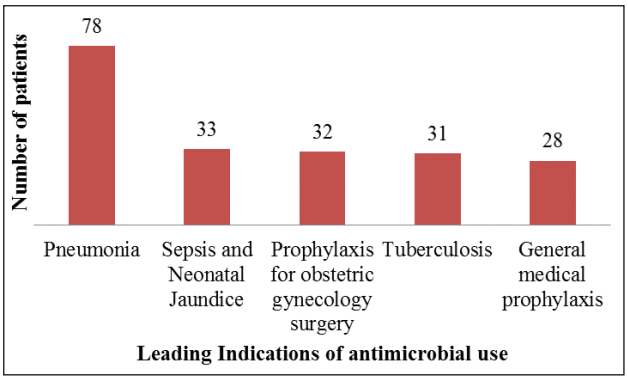


Figure 3. Indications for antimicrobial agents in Mbagathi Hospital.

Culture and sensitivity testing

There were only 7 (3.8%) culture and sensitivity requests. The highest number of requests (4, 57.1%) came from nursery. Medical and maternity wards did not have any requests. Out of the seven requests, results were available for only 4 requests. The other three were still being processed.

Compliance with WHO indicators and specific guidelines for antimicrobial prescribing

Table 4. Compliance with WHO indicators for antimicrobial prescribing at Mbagathi Hospital

Measure	Indicator	Mbagathi Hospital score	WHO optimal values
Extent of antimicrobial use	Percentage of prescriptions with an antimicrobial	78.9%	<30%
Polypharmacy	Average number of antimicrobials per encounter	1.96	1.3-2.2
Compliance to generic prescribing	Percentage of antimicrobials prescribed by generic name	80%	100%
Guideline compliance	Proportion of patients who received surgical prophylaxis as per guidelines	0.0%	>70%
	guideline Proportion of patients with pneumonia who received antibiotic treatment as recommended in the treatment	40%	>70%
	Proportion of patients who received second line antimalarials after a positive malaria test	90%i	100%
	Percentage of drugs prescribed from the essential drugs list	100%	100%
Irrational use of surgical prophylaxisn	Percentage of encounters with Surgical prophylaxis exceeding 24 hours	100%	_
Prescribing errors	Frequency not indicated on prescription Route not indicated Duration of use lacking No stop review for antimicrobial Missed doses during course of treatment	2.2% 0.8% 53.2% 39.1% 45.5%	Errors should be avoided 100% of the time

Nearly 80% of patients studied were on one or more antimicrobials. The reference WHO value is 30% hence antimicrobial use was quite high in this study. The average number of antimicrobials prescribed was approximately 2 which is within the reference range. Proportion of medicines prescribed by generic name was 80%. Almost 50% of patients missed one or more doses during their course of treatment. Over 50% of prescribed antimicrobials did not have duration of use specified.

Linear regression for risk factors for number of antimicrobials prescribed per patient

Bivariable linear regression was carried out by regressing the number of antimicrobials prescribed against each of the covariates. A parsimonious model of the most important predictors of number of antimicrobials prescribed was also conducted. The co-efficients showed that the most important determinants of the number of antimicrobials prescribed were HIV status, nutritional status, presence of catheterization and previous hospitalization. These variables were retained in the most parsimonious model. The most powerful predictor for number of antimicrobials prescribed was HIV status with adjusted β co-efficient of 2.187 as shown in table 5.

Table 5. Linear regression for risk factors for number of antimicrobials prescribed per patient.

Variable	Crude β coefficient		Adjusted β coefficient	
	β (95% CI)	p-value	β (95% CI)	p-value
HIV status	2.867 (2.436 - 3.297	<0.001	2.187 (1.617 - 2.759)	<0.001
Catheterization	2.089 (1.740 - 2.438)	<0.001	1.317(1.055 - 1.580)	<0.001
Previous hospitalization in the last 90 days	1.307 (.848 - 1.766)	<0.001	0.516 (.183 - .850)n	0.003
Nutritional status	1.510 (1.101 - 1.920)	<0.001	0.264(-.0314 - .560)	0.080
Age group	-0.124 (-.294 - .0460)	0.152	----	---
Ward type	0.642 (-.087 - .215)	0.402	---	---
Intubation	0.137 (-.862 - 1.135)	0.787	---	---
Sex	-0.026 (-.480 .428)	0.91	---	---

Discussion

This survey found that 79.8% of patients admitted in Mbagathi hospital received one or more antimicrobials. This is comparable with a previous study done in Kenyatta Referral Hospital in Kenya [11]. Similar outcomes were observed in a study done in Ethiopia [12]. However, hospitals

in Ghana and South Africa had lower antimicrobial use prevalences [13]. In this study the most important risk factor for number of antimicrobials prescribed was HIV status. The others were previous hospitalization, catheterization and nutritional status. In point prevalence survey in Botswana some risk factors associated with number of antimicrobials used included age-group, prior admission, referral from another facility, being malnourished, having tuberculosis and HIV infection [14].

Prior antimicrobial use was noted in 45(24.3%) participants. The bulk of these patients were in paediatric ward and this was attributed to thorough history taking of prior use. It could also be attributed to increased prevalence of parents self-medicating their children. This proportion could have been actually higher if all wards routinely sought this information from patients. The commonest antimicrobial among the adults was co-trimoxazole while amoxillin was commonest among the children. This corroborates with other studies in Accra Ghana and United Arab Emirates. [15]. In a point prevalence study in Botswana patients had prior exposure in the last 90 days to cefotaxime at and amoxicillin [14]. This is in tandem with our study whereby amoxicillin was widely used pre-admission. Self-medication especially with amoxicillin has led to extensive antimicrobial resistance.

A total of 363 antimicrobials were prescribed during hospitalization. Antibiotics formed the biggest proportion of antimicrobials prescribed followed by antivirals, antimalarials and antifungals. The bulk of prescribed antivirals were antiretroviral drugs. Similar results were obtained in another study conducted among 53 countries worldwide, 41,213 antimicrobials were prescribed whereby antibacterials constituted 36,792 (89.3%), and both antimalarials and antifungals were 1,724 (4.2%) [16]. Results from the first global point prevalence study showed that out of 48,565 antimicrobials prescribed, 43,513 (89.6%) were antibacterials, 2,062 antifungals for systemic use [17].

In our study, third generation cephalosporin ceftriaxone topped the list of prescribed antimicrobials. These values are consistent with reports in the literature. In a study conducted at Kenyatta referral Hospital, Kenya, ceftriaxone was the commonest antimicrobial prescribed [11]. Comparable findings were seen in a retrospective observational analysis of antimicrobials [18] where ceftriaxone was the most common prescribed antimicrobial of the time. In the contrast in an Ethiopian study, the most prescribed antimicrobial was penicillin G crystalline [19]. Cephalosporins particularly third generation are very popular. They have wide spectrum of activity, minimal toxicity, are easy to administer and readily available as well. In an antimicrobial use review by Verspoten et al; [16], vancomycin and carbapenems were highly utilized in both North and Latin American hospitals unlike our study where they were minimally used. Reasons could be high prevalence of methicillin resistant Staphylococcus aureus (MRSA) in Latin American Hospitals [16]. The high cost of these antibacterials is also an inhibition to their use especially in low and middle income countries like Kenya. Most studies on antimicrobials in India have found a high prevalence of ceftriaxone use [20]. In a study conducted in a Kenyan referral hospital ceftriaxone was the most common prescribed antibiotic [21]. Overuse may be attributed to non-adherence to guidelines as well as a weak medicines and therapeutics committee to reinforce good prescribing practices. The implications of overuse of ceftriaxone are dire. With the dwindling pipeline of new antibiotics and growing risk of resistance to ceftriaxone mortality rates from simple infections is expected to increase. Empowering the hospitals medicines and therapeutics committee as well as establishing an antimicrobial stewardship programme (ASP) might help to curb this menace. Other point prevalence surveys are suggested in future to compare and evaluate impact of established ASP.

Several antimicrobials accounting for 22.6% did not have accompanying duration of use in the treatment sheet. In a study conducted in Uganda, the prescriber omitted duration in most prescriptions [13]. A stop review date was available for 60% (221) of the antimicrobials but 40% (142) had no indication on when to stop or review treatment. This means that the nurses will administer them daily until discharge. This is serious misuse and leads to high costs and resistance.

A big proportion of patients 45.5% missed at least one dose during their hospitalization with some missing up to 30 doses. Missing doses aggravates resistance since efficacy is already compromised. This is comparable to a study done among hospitalized patients in Uganda where almost half of patients missed at least one dose of their antimicrobial treatment [13]. In Botswana, 1923 doses from 437 prescriptions failed to be administered, with a mean of 1.96 doses [14]. Missed doses may have been occasioned by some system related problems including stock-outs, understaffing especially since most were parenteral and a nurse is needed for administration and poor communication between the health care providers and the patients.

In our study the most common indication was pneumonia (n=76) followed by neonatal sepsis and neonatal jaundice (n=33), prophylaxis for obstetric gynecology surgeries third (n=32), tuberculosis (n=31) and general medical prophylaxis (n=28). Similar findings in Ethiopia were reported indicating pneumonia and sepsis as top indications where ceftriaxone was indicated [22]. Similarly, in an internet based study among 53 countries pneumonia was the commonest overall indication of patients treated [16]. However, in a survey at Kenyatta referral hospital, Kenya, the most prevalent indication was medical prophylaxis [11]. Contrary to our study, in a point prevalence survey in Ethiopia, the biggest indications were associated with obstetrics and gynecology [14]. Out of 146 antimicrobial episodes observed, 86(59%) were for prophylaxis. Medical prophylaxis constituted 44(51.2%) while surgical prophylaxis was at 42(48.8%).

Out of the sampled 185 records there were only 7 culture and sensitivity requests (3.8%). In a Ghanaian study only 14 out of 382 patients on antibiotics had a biomarker test done

[23]. Empirical antibiotic use was very common and clinical judgment was very rampant. Laboratory utilization for microbiology is very limited at this facility. This may have been due to unavailability of the services, delays in processing results, clinical suspicions of septicemia warranting immediate antimicrobial use.

Strengths and Limitations of the Study

The use of a standardized protocol is a big strength for this study. This allowed for comparability nationally and internationally.

Being a retrospective study it had some limitations. The study was done over three weeks therefore a different pattern could also have been noted had the period been prolonged over other months. It did not measure severity of illness hence it was not feasible to relate drug use patterns with severity of patient's sicknesses. The quality of the records was also poor and lots of observations had to be done since the study protocol did not allow for interviews.

Conclusion and recommendations

Prescribing practices were poor as a big proportion of antimicrobials did not have a stop preview, missed indication; had no frequency and most antimicrobials prescribed were not administered at all. Culture and sensitivity was not utilized to guide patient care. Patient management was largely empiric.

Recommendations for practice

A hospital antimicrobial stewardship committee is recommended if not in place or activated if already in place. The committee should henceforth make it policy to conduct culture and sensitivity testing before commencing antimicrobial therapy. Continuous medical education sessions touching on proper prescribing habits need to be emphasized. This includes prescriber's sensitization to indicate antimicrobial treatment frequency, duration and stop review. Dangers of missed doses should be emphasized. Attention could also be directed on facilitating intravenous to oral switch of antimicrobials as well as focus on improving adherence to surgical prophylaxis guidelines. This should form the orientation package for all interns and newly employed health care workers.

Conflict of interest

The authors declare that they have no competing interests.

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Author's contributions

All authors helped to conceptualize the project, background information, data analysis and guided on the methods and results sections. They all read and approved the final manuscript.

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Inactive Ingredients used in Alcohol-Based Hand Sanitizers marketed in the Nairobi Metropolitan Area

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Abstract

Background: Alcohol-based hand sanitizers (ABHS) have become widely used products since the advent of the SARS-CoV-2 coronavirus based COVID-19 pandemic. In addition to ethanol or isopropanol (the active ingredients of ABHS) and water, these products are formulated with a number of ingredients to optimize delivery, efficacy and safety as well as to provide consumer appeal. Despite the widespread use of ABHS, there is very limited information in the literature on the non-alcohol ingredients used in these products.

Objectives: The aim of this work was to determine the inactive ingredients used in ABHS marketed in metropolitan Nairobi.

Methodology: ABHS products were randomly obtained from several locations at retail outlets within the Nairobi metropolitan region. The ingredients used in each ABHS were obtained from the product labels.

Results: The most common inactive ingredients based on percentage frequency of listing on product labels were glycerin (50%), fragrances (36%), carbomer (26%), triethanolamine (18%) and propylene glycol (17%). It was observed that some products incorporated additional antimicrobial agents and preservatives in the formulation. The fragrances and some of the preservatives used in the ABHS products are potential allergens. Incomplete or inadequate ingredient naming was noted for several products.

Conclusions: There is a need for ABHS manufacturers to fully disclose all raw materials used in ABHS products using standardized ingredient nomenclature. ABHS users need to be aware of potential allergens present in respective marketed products.

Key Words: *alcohol based hand sanitizers, hand rubs, inactive ingredients, allergens, COVID-19.*

Introduction

Inactive ingredients (excipients) are critical components in the formulation and usage of hand sanitizers [1].The active ingredients in hand sanitizers are antimicrobial compounds such as alcohols (ethanol or isopropanol) or benzalkonium chloride [2,3]. Only alcohol-based hand sanitizers (ABHS), however are currently recommended for hand disinfection against SARS-CoV-2, the causative agent of COVID-19. Excipients are added to hand sanitizers to facilitate their handling, application, reduce adverse dermatological effects and enhance marketing appeal. Examples of excipients used in hand sanitizers include viscosity enhancing agents (thickeners), pH adjusting agents, humectants, fragrances and coloring agents [4].

Following the emergence of the coronavirus pandemic, numerous ABHS became available with many new entrants seeking to take advantage of the high profit margins and low market entry barriers associated with the manufacturing of these products. Early work by Nyamweya and Abuga showed that many ABHS being sold in Nairobi and its environs did not meet established regulatory standards for labelling with some products appearing to have formulation issues [4]. Other than for glycerin (glycerol), there are very few publications in the literature regarding the inactive ingredients used in commercially available ABHS. The WHO based formulations use an alcohol, water, hydrogen peroxide and glycerin [5]. A recent study in South Korea by Yoon et al. evaluated the ingredients in thirty-four ABHS [6]. Excluding alcohol and water, the most common ingredients (present in at least 10% of products) were carbomer, extracts (type not specified), glycerin, triethanolamine, oil, perfume, propane-1,2-diol (propylene glycol), tocopheryl acetate, butylene glycol, aminomethyl propanol and polysorbate. Voller et al., reported on the occurrence of allergenic ingredients found in hand sanitizers (both ABHS and non-ABHS) at several major hospitals in the United States [7]. Given that inactive ingredients may influence the efficacy of ABHS as well as, in some cases, be potential allergens, further work on profiling the ingredients used in ABHS is needed.

Objectives

The objectives of the study were to identify the non-alcohol (i.e., non-ethanol and non-isopropanol) raw materials used in ABHS products marketed in the greater Nairobi region and to determine the frequency of use of each ingredient. Additionally, the work sought to categorize these ingredients by their functionality and to identify allergenic materials in the formulations.

Methodology

Sixty-six ABHS products available in the market were randomly purchased from various retail outlets in metropolitan Nairobi following the onset of the COVID-19 pandemic as described in an earlier publication [4]. The ingredients used for each ABHS were recorded from the manufacturer's labelling. The information was entered into a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA) for data analysis. Allergenic ingredients were identified based on their listing in the American Contact Dermatitis Society (ACDS) 2017 Core Allergen Series [8].

Results

The frequency of occurrence of ingredients in the ABHS is shown in Figure 1. Twenty-seven percent of the ABHS products did not list their inactive ingredients which is a regulatory and safety concern.

A total of forty ingredients (excluding the active alcohols and water) were identified from the product labels of the various ABHS products. The number of specific ingredients however, may be higher as some product labels used general terms such as emollient, emulsifier, moisturizer, preservative, solubilizer and thickener in their ingredient lists. Furthermore, non-specific names such as colors, copolymer and essential oils were also used for some products.

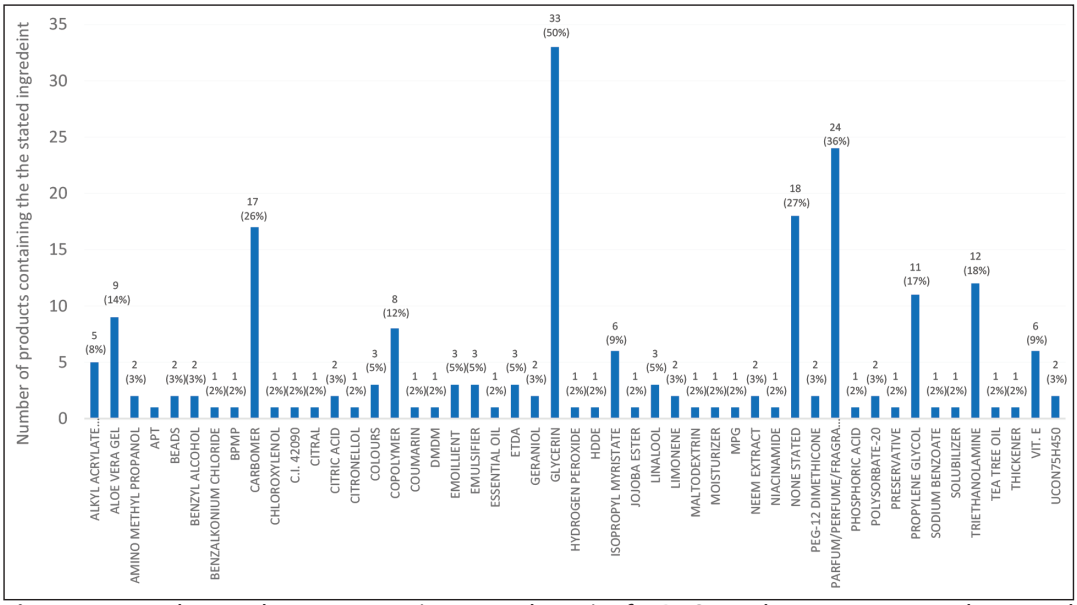

Figure 1. Number and percentage (in parentheses) of ABHS products containing the stated ingredient. Abbreviations: APT – ammonium polyacryloyldimethyl taurate; BPMP – butylphenyl methylpropional; HDDE – hydroxydichlorodiphenyl ether; T.E.A. – triethanolamine; Vit. E –Vitamin E/tocopheryl acecate.

The inactive ingredients used in the ABHS products are summarized in Table 1 by functional class. In addition to functionality, factors which play a role in manufacturer selection of raw materials are compatibility (non-reactivity with other ingredients and the primary packaging), price, availability and regulatory status.

Table 1. Classification of inactive ingredients listed in ABHS products

Functional Class	Ingredients specified in the ABHS Products
Humectants and moisturizers	Aloe vera gel, glycerin, propylene glycol*
Emollients	Dimethicone, isopropyl myristate, jojoba ester, UCON75H450[b]
Thickening agents	Alkyl acrylate crosspolymer, ammonium polyacryloyldimethyl taurate, carbomer
pH adjusting agents	Aminomethyl propanol, citric acid, phosphoric acid, triethanolamine
Antimicrobials and preservatives	Benzyl alcohol*, benzalkonium chloride*, chloroxylenol*, DMDM hydantoin*, hydrogen peroxide, hydroxydichlorodiphenyl ether, sodium benzoate*
Antioxidants and stabilizers	EDTA [c], tocopheryl acetate*
Surfactants	Polysorbate-20
IFragrances	Butylphenyl methylpropional, citral, citronellol, coumarin, geraniol, linalool, limonene, (other products list parfum, perfume or fragrance)*
Colors	C.I. 42090†. All others listed as "colours" only
Other[a]	Beads, maltodextrin, neem extract, niacinamide, tea tree oil*

[a] Reasons for specific use in ABHS not established; [b] Polyethylene glycol/ Polypropylene glycol-17/6 copolymer; [c] Ethylenediamine tetra-acetic acid.* potential allergen based on American Contact Dermatitis Society (ACDS) 2017 Core Allergen Series [8]. †Brilliant blue FCF

Discusion

The labelling of ingredients used in the manufacturing of pharmaceuticals and cosmetics is considered to be a good practice for which guidelines have been established [9,10]. This information is especially important for individuals who need to avoid certain chemicals due to sensitivities and allergies. Therefore, manufacturers need to ensure that ingredients are properly listed to permit their identification by ABHS users. In some of the products sampled in this study abbreviations, such as DMDM, IPM and MPG were used. These abbreviations presumably refer to dimethylol dimethyl hydantoin, isopropyl myristate and mono-propylene glycol respectively. However, ideally, the full names of each ingredient should be used in the label to avoid ambiguity.

Excluding ethanol, isopropanol and water, the number of

ingredients used per product ranged from one to thirteen with an average of four ingredients per ABHS product in the current study. The five most common inactive ingredients and their percent frequency of occurrence were glycerin (50%), parfum/fragrance (36%), carbomer (26%), triethanolamine (18%) and propylene glycol (17%). Glycerin and propylene glycol are used as humectants to alleviate the drying effects of alcohols in the skin; fragrances provide aesthetic appeal; carbomers are polymeric thickeners which facilitate ABHS dispensing and application; and triethanolamine is used to optimize pH for the carbomers.

Other excipients which were observed frequently (present in at least 10% of the ABHS products) were aloe vera gel (14%) and copolymer (12%). Aloe vera is a multifunctional ingredient which is often used in topical formulations as a moisturizer [11], as well as to enhance marketing appeal. The copolymers are most likely used as viscosity enhancing agents.

In comparison, the aforementioned work by Yoon et al., found that carbomer was used in all the ABHS products studied [6]. The percent frequencies of the other most common ingredients in their study were extracts (94%), glycerin (65%), triethanolamine (53%), oil (29%), perfume (26%), propylene glycol (26%), tocopheryl acetate (24%), butylene glycol (21%), aminomethyl propanol (15%) and polysorbate (12%).

Potential allergenic ingredients are indicated with an asterisk in Table 1. In contrast to the study by Voller et al. where the most frequent allergens were tocopherol (51.3%), fragrance (40.0%), propylene glycol (27.5%), benzoates (25.0%), and cetyl stearyl alcohol (12.5%) [7], in the current study only fragrances and propylene glycol had a frequency of occurrence in more than 10%. Fragrances are of particular concern as they are potential allergens that may lead to contact dermatitis. Their use, however, is common in ABHS to mask the characteristically strong alcohol odor, which some users may find unpleasant. Marketing appeal and product branding are also a major reason for the use of fragrances.

The functional classes with the greatest variety of ingredients were the antimicrobial/preservative and the fragrance categories each with seven ingredients. Fragrances and preservatives have been frequently reported to be the most common types of allergens in cosmetic products [12,13].The use of antimicrobial and preservative agents is surprising given that the active ingredients in ABHS, namely ethanol or isopropanol, already have a broad range of antimicrobial activity. While non-volatile antimicrobial agents have the advantage of providing prolonged residual antimicrobial action [3,14], their use in combination with alcohols may not necessarily result in improved efficacy [15,16].

None of the products used the WHO recommended hand sanitizer formulations [5]. The use of a range of ingredients suggests that local manufacturers prefer to develop their own ABHS formulations.

The use of humectants, moisturizers and emollients (skin softening agents) indicate that dermal effects are an important consideration for ABHS products. Humectants and moisturizers are added to counteract the drying effect of alcohols on the skin, which may with frequent use compromise its barrier function thereby predisposing the user to skin irritation or contact dermatitis. In contrast emollients are used to soften and condition the skin. Some of these agents may also improve the sensory characteristics of the ABHS product during application onto the skin.

Conclusion

There is a need for manufacturers to ensure that all the ingredients in ABHS products are accurately listed using names that conform to established international ingredient nomenclature standards. The use of non-specific names, terms or abbreviations for ingredients used in these products should not be permitted. In addition to complying with good manufacturing practices, this will enable users of these products to make informed decisions which are especially critical for persons who need to avoid certain ingredients for medical or personal reasons. Consumers prone to contact dermatitis and allergic reactions should be aware of the widespread use of fragrances in ABHS products.

Conflict of Interest

No conflict of interest is declared by the authors.

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Antimicrobial nanoparticles and their application in medicine

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Abstract

Diseases caused by micro-organisms have become a risk to human health worldwide due to the extra and inappropriate uptake of antibiotics. This has led to antimicrobial-resistant microbes that resist clinical treatment. The resistance could be due to several mechanisms such as the microbe forming a biofilm, overexpressing efflux pumps to expel the drugs, modification of the drug, or the drug target. The search for safe and alternate novel antimicrobial agents which can treat these infections is very important if we have to overwhelm the resistant micro-organisms. Nanotechnology has shown the potential to fight infectious organisms. Due to their size, nanomaterials can improve cellular uptake of drugs and can also be conjugated with ligands, for example, peptides and antibodies, for objective therapeutics. To avoid drug toxicity, drugs can be encapsulated in nanomaterials (N.M.s) for sustained release. In this work, we present a systematic review of the literature describing the recent developments in the field of antimicrobial N.M.s, their synthesis, mode of action, and application in diagnosis, treatment, drug delivery, and vaccine development. The review was done via various search engines using relevant keywords.

Key words: *Microbial infections; Antimicrobial nanomaterials, nano-diagnosis, therapeutics.*

Introduction

Worldwide, the growth and spread of antimicrobial resistance (AMR) are known to endanger many organisms including humans and animals [1]. AMR is the evolvement of micro-organisms over time to develop the ability to resist antimicrobial therapies such as antibiotics. The quick emergence of multi-drug resilient pathogens is largely attributed to the abuse and incorrect intake of antibiotics [2]. Though several scientists have continued with studies into the advancement of novel antibiotics, they have not been able to cope with the fast growth of bacterial resistance [3]. Due to this, the growth of this antibiotic resistance might be experienced quicker than the development of newer agents for the treatment of these infections. It is therefore important to develop a superior and clear understanding of antimicrobial resistance mechanisms [2].

Antimicrobial resistance occurs mainly at the gene or protein level due to various factors such as gene mutations and the attainment of foreign DNA coding [4]. The acquired resistance genes exist in plasmids, transposons, and integrons and are transferred to the bacteria through transformation, transduction, or conjugation [5] as has been demonstrated for *Escherichia coli , Klebsiella pneumoniae*, and even *Pseudomonas aeruginosa* (6). It has been reported that Methicillin-Resistant Staphylococcus aureus (MRSA) is unaffected by virtually most of the β-lactam antibiotics [7] causing many infections due to the production of a penicillin-binding protein with significantly decreased binding affinities to β-lactam antibiotics [8].

At the protein level, microbes may produce enzymes such as lactamases that destroy the antimicrobial agent or inactivate the molecule by adding modification groups such as phosphate, acetate, or adenylate [4]. Such modifications render the antimicrobials unable to interact with their target. Biofilm formation as illustrated in figure 1 [9] has also been attributed to increased antimicrobial resistance. The films protect the covered cells against altered pH, osmolarity, nutrient scarcity, mechanical shear, and block access by the antimicrobials and host immune cells [10]. The increasing resistance of gram-negative K. pneumonia, has been associated with its ability to grow as a biofilm [11].

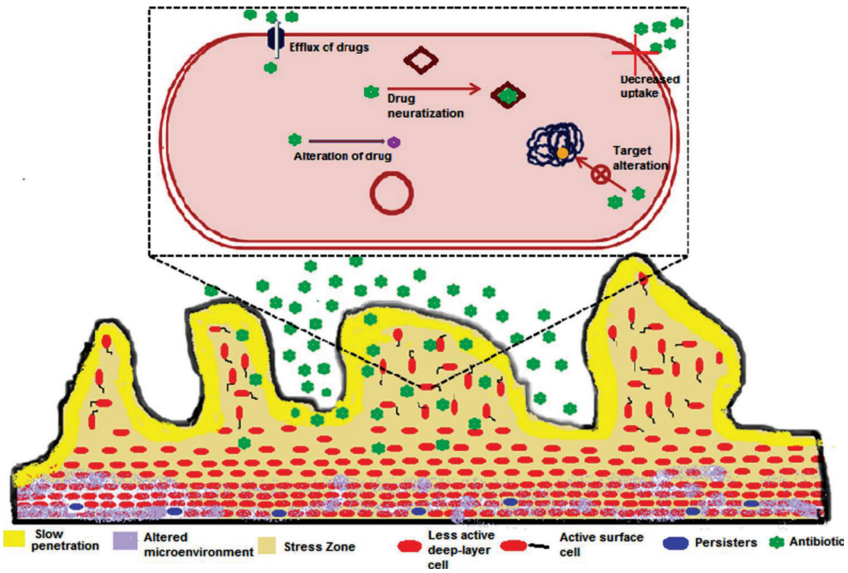

Figure 1. A schematic illustration of the possible mechanisms of antibiotic resistance in the communities of biofilms. Reproduced from an open-access article [9].

The best method reported controlling this antimicrobial resistance crisis has been the development of newer approaches such as combining antimicrobial drugs with additional agents leading to the neutralization and impediment of the antimicrobial-resistant mechanisms conveyed by the pathogens [12].

The advancement of nanotechnology is attracting significant consideration due to its countless advantages in the pharmaceutical field [1] such as providing innovative and robust detection tools for pathogens as well as prompt and sensitive characterization of molecular biomarkers of drug resistance [13]. It has led to the development of N.M.s, sized between 1 and 100 nm, with antimicrobial properties such as the interference of the cellular membrane, dispersion into and degradation of internal cellular components, and the release of ions that have antimicrobial activity, thus emerging as potential substitutes to traditional antimicrobial therapies [14]. Numerous types of antimicrobial nanomaterials such as nanoparticles due to their unique properties have demonstrated great efficacy for the management of antimicrobial-resistant diseases, hence offering a better therapy than conventional drugs [15]. It has been reported that metal N.M.s and their derivatives, including silver nanoparticle (AgNPs), silver oxide (Ag2O), gold nanoparticles (AuNPs), copper nanoparticle (CuNPs), and metal oxide N.M.s including copper oxide and zinc oxide reveal an extensive variety of antimicrobial activity against diverse species of micro-organisms [16]. Since the N.M.s' have direct contact with the cell wall of the bacterial, and they do not need to infiltrate the cells, this might mean that the N.M.s would be less susceptible than conventional antimicrobials to stimulate bacterial resistance [17]. This review presents relevant and recent literature describing N.M.s that have demonstrated antimicrobial activity, providing an overview of the properties and synthesis of antimicrobial N.M.s as well as their applications in medicine.

Synthesis of antimicrobial nanomaterials

Nanomaterials (N.M.s) are mainly synthesized via the top-down and bottom-up which normally differ in terms of the methods used to assemble the N.M.s [18, 19]. In the "top-down" approach, particles are fabricated following the deconstruction of bulk materials into smaller fragments, typically by physical and chemical reactions [19] including methods such as lithography etching [18, 20], laser ablation, chemical etching [18, 21], beam milling process materials [22], and thermal decompositions [23], which have been used to synthesize novel N.M.s such as nanowires, magnetic iron oxide nanoparticles [19, 24].

The "bottom-up approach," includes assembling atoms and their precursors into nanostructured arrays via chemical reduction processes [19, 25]. Usually, reducing agents such as sodium citrate reduce the metal ions to atoms or molecules which then gather together to form crystals that then grow larger [26]. Capping agents and stabilizing agents are then added to stop the growth of the crystals and control the size of the N.M.s [19, 27]. The self-assembly of the atoms or molecules makes this approach generate functional multi-component devices with minimal wastage [19, 28]. The most common "bottom-up" synthetic methods include chemical Vapor Deposition CVD [19, 29], sol-gel process [19, 28], laser pyrolysis, and green methods [19, 30].

Characterization of antimicrobial nanomaterials

Microstructural characterization of N.M.s is fundamental since it provides information about the different aspects such as shapes, sizes, morphology, and certain defects among others [31, 32]. The most common characterization techniques used include transmission electron microscopy (TEM) [33], Fourier transforms infrared [FTIR], scanning electron microscopy (SEM) [34], atomic force microscopy (AFM), ultraviolet-visible spectroscopy (UV-Vis), among others.

Antimicrobial mechanism of nanomaterials

Depending on their class, antibiotics kill or inhibit bacterial growth by stopping the Synthesis of proteins and metabolites, disrupt binary fission, or damage the integrity of the cell wall/membrane [35]. Regrettably, microbial resistance may develop against each one of these modes of action. There are different types of antimicrobial classes based on the mechanism of action. For example, Penicillins inhibit the synthesis of the cell wall while Lipopeptides depolarize the cell membrane. Other antimicrobial groups such as Tetracyclines and Macrolides inhibit the synthesis of proteins while quinolones inhibit the synthesis of nucleic acids.

The N.M.s are known to have the potential to fight disease-causing pathogens due to their many unique properties [36] such as their fast and rapid synthesis [37]; their ability to solubilize and stabilize drugs [38]; and their biocompatibility with target agents enabling them to be controlled by light, pH, and heat [39]. They have very small sizes and huge surface-to-volume ratios that give them their distinct functionality in drug delivery, making them competitive compared with the conventional therapies used to treat pathogenic infections [16]. Although the efficiency of various antibiotics is limited by reduced membrane transport [40], loading the drugs with nanoparticles can enable them to get into host cells through endocytosis, assisting their intracellular access [39]. Co-administration of protein-based drugs can also be improved by interacting surface lipids with nanoparticles such as AuNPs, enhancing membrane penetration [41].

Several mechanisms by which N.M.s exert their antibacterial activity have been reported in the literature. For example, as represented in figure 2 [36], N.M.s can interact with the bacteria cell wall directly (42); (ii) they can obstruct biofilms formation (iii) activate the innate and the adaptive host immune responses; (iv) encourage oxidative stress and (v) induce DNA and/or proteins interactions [43]. Normally, these mechanisms are not similar for all N.M.s, and hence they can be of extreme importance in the fight against MDR bacteria [44]. The ROS, which are natural byproducts of cellular oxidative metabolism, supports the antibacterial activity of the many N.M.s by damaging and destroying the

cellular components of the pathogens resulting in cell death [45]. The direct contact of N.M. with the microbe cell wall, without penetrating the cells like standard antibiotics, can be used to overcome various conventional antimicrobial resistance mechanisms, including resistance due to biofilms formation [36].

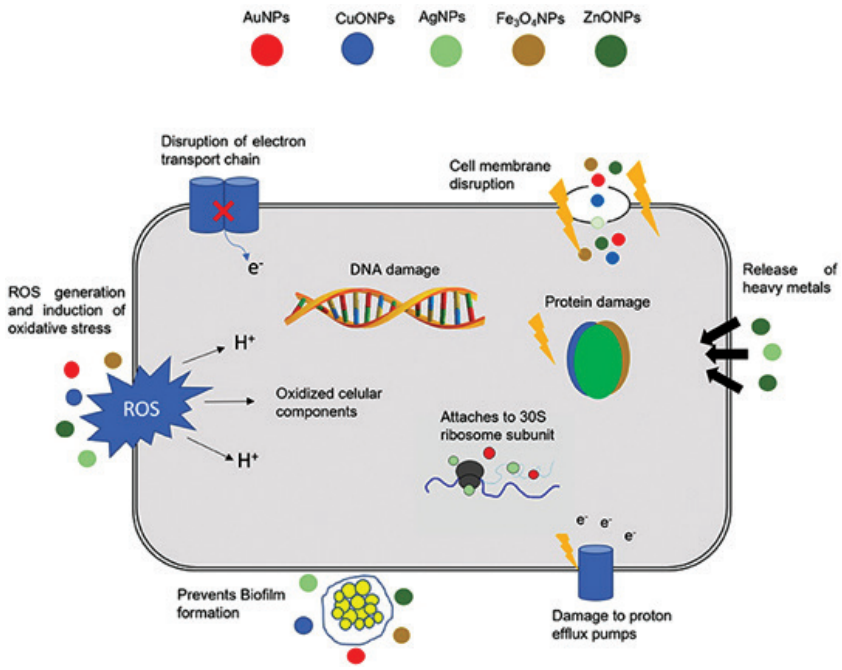

Figure 2. A schematic diagram showing the different N.P.s modes of action in bacterial cells. Reproduced with permission from [36].

Application of antimicrobial nanomaterials in medicine

N.M.s have an incredible potential to offer a solution for numerous issues, especially in medicine. This is because most N.M.s, such as N.P.s, can infiltrate the cell membrane of pathogenic micro-organisms, which interferes with key molecular pathways, expressing exceptional antimicrobial mechanisms [36, 42]. The combination of N.P.s with optimal antibiotics has shown synergistic effects that might help control the global crisis of developing bacterial resistance [42]. The following sections discuss the various applications of antibacterial N.M.s in medicine specifically, in microbial infection diagnosis, microbial infection treatment, drug delivery, and vaccines. The antimicrobial mechanisms of the N.M.s responsible for each application are also highlighted.

Antimicrobial nanomaterials in the diagnosis of microbial infections

Valuable diagnostic tools for infectious diseases include polymerase chain reaction (PCR) and Enzyme-linked immunosorbent assay (ELISA) [46]. However, these methods are tiresome, costly, insensitive in some cases, and normally require trained personnel [47]. Using specific target sequences to identify pathogens can distinguish between organisms and characterize detailed distinctions at the genomic level providing excellent nucleic acid signatures that are suitable for diagnostics [13]. Nanomaterials have greatly enhanced the sensing of bio-molecular interactions by improving the sensitivity and detection limits of optical, electrical, and electrochemical biosensors due to their small sizes [48]. Effective novel strategies to detect microbial pathogens might be promising since they may allow early diagnosis and timely treatment of microbial infections [49] hence improving human health. These N.M.s have been incorporated in the development of biosensors for the detection and diagnosis of microbial toxins, bacterial and viral agents, and proteins and nucleic acids [48]. They have also been used to develop nano-diagnostic assays point-of-care, and economical detection of microbial agents [47]. Several N.M.s such as gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), magnetic nanoparticles, and carbon nanotubes (CNTs) have been used as illustrated in figure 3 [46].

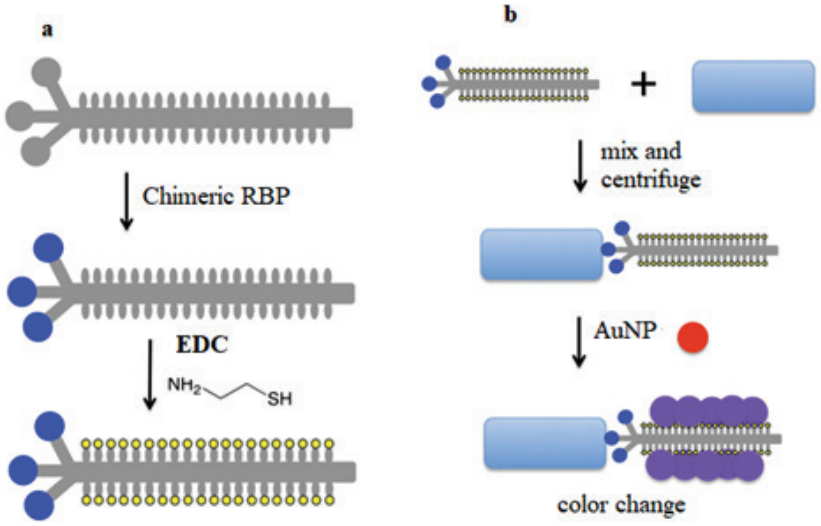

Figure 3. A schematic diagram representing the chimeric phage detection of bacterial species. Adapted with permission from [46].

Antimicrobial nanomaterials in the treatment of microbial infections

N.M.s have shown the potential to be used to fight deadly infections [50] owing to their unique properties. They have been reported to have enhanced influence on a given micro-organism and other diseases [51, 52]. N.M-based strategies have shown the ability to overcome the obstacles faced by traditional antimicrobials, such as antibiotic resistance mechanisms including overexpression of efflux pumps [50]. This enables them to achieve numerous new

bactericidal pathways to attain antimicrobial activity [50, 53] or act as nano-carriers as illustrated in figure 4 [54].

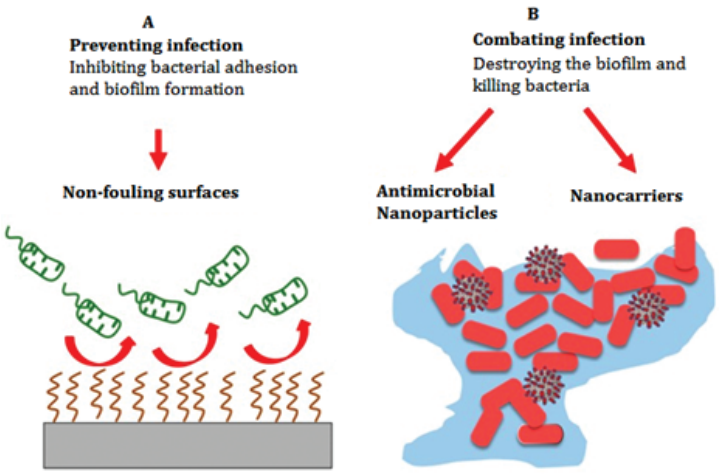

Figure 4. A schematic diagram representing (a) The inhibition of the bacterial adhesion via surface modification; (b) the use of antibacterials nanoparticles and nano-carriers to destroy the formed biofilm. Reproduced from [54], an open-access article.

Silver nanoparticles (AgNPs) are potent antibacterial agents [55] through multidimensional modes of action including microbial cells binding and cells infiltration leading to cell death among others [55, 56]. Data from several studies have indicated that AgNPs have been used alone or in combination with antibiotics to fight against microbial infections [55, 57], sometimes exhibiting advanced antimicrobial activity than antibiotics [58].

Several reports show that low concentrated, AuNPs are usually not antibacterial but experience weakly antibacterial activity at high concentrations, probably due to the effects of co-existing chemicals involved in their syntheses but not completely removed [36, 59]. However, several studies have associated the antibacterial mechanism of AuNPs with either the hindering of the ATPase activity by the membrane potential collapse as well as deterring the binding of the ribosome subunits to tRNA [60] or the AuNPs attacking the nicotinamide thus affecting the bacterial respiratory chain [61]. Furthermore, AuNPs and Au nanoclusters have catalytic activities comparable to several enzymes. This enables them to generate ROS affecting the bacteria through oxidative stress mechanisms [62]. Also, a novel nanoformulation containing Au nanorods in combination with near-infrared (NIR) photothermal treatment was reported to show remarkable antibacterial efficacy in treating *Pseudomonas aeruginosa* infection in drug-resistant pneumonia [63]. Reports show that conjugating AuNPs to antibiotics, like vancomycin and methicillin, etc., escalates their intrinsic activity against MDR strains [64, 65]. Other nanomaterials such as metal oxide nanomaterials [66, 67] and carbon nanotubes [68, 69] have also been reported to have antibacterial effects.

Application of antimicrobial nanomaterials in drug delivery

Nanoparticles (N.P.s) can act both as antibacterial agents as well as nano-carriers for antimicrobial molecules [70]. In most cases, observed antibacterial properties arise from the antibiotic molecule binding to the surface of the N.P.'s or captured inside and conveyed to the microbe via their direct interaction [71]. Various N.P. constructs have been investigated for drug delivery such as polymer-based N.P., liposomes, dendritic polymers, inorganic metal nanoparticles, nanocrystals, and inorganic non-metallic N.M.s as illustrated in Figure 5 [72] (See below).

Depending on the polymer, polymer-based N.P.s can encapsulate drugs within the nanoparticle cavity or be attached to its surface by the polymerization and released by desorption or diffusion in the target tissue [71, 73]. Polymers such as chitosan, alginate, and collagen, as well as polylactic acid, have been used. For example, polymeric N.P.s can surround hydrophilic or hydrophobic drug molecules and macromolecules such as nucleic acids [71, 73]. Likewise, liposomes can carry both hydrophobic and hydrophilic compounds without chemical modification due to their unique structure. Following modification using components such as polyethylene glycol, the lipid bilayer can improve the liposome's half-life [71, 73]. A drug can be released from the liposome by changing parameters such as pH and osmotic gradient. Dendritic polymers are highly branched molecules that provide a three-dimensional architecture with void crevices in the internal part, and many functional branches grow radially from the center. These properties make it possible to either encapsulate a drug within the interior cavity or the highly

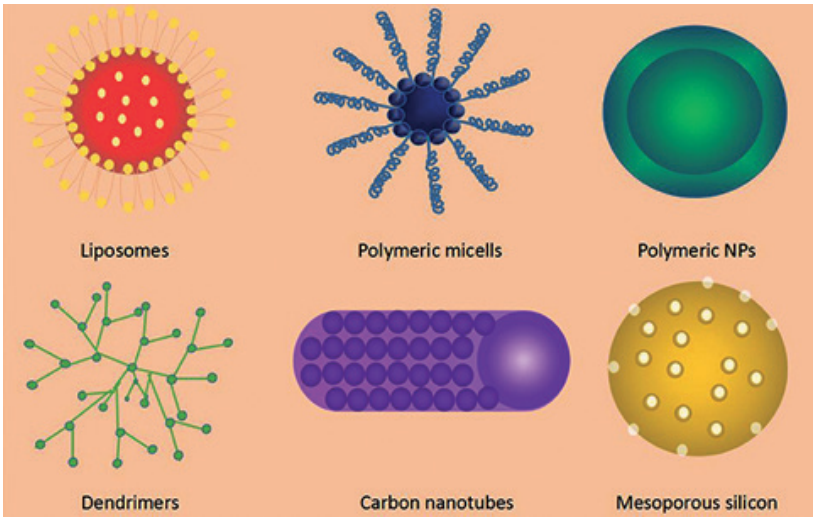

Figure 5. Common types of nano-drug carriers. Reproduced from [72], an open-access article.

symmetric branched outer layer through either covalent bonding or noncovalent complexation [71].

Delivering antibiotics using N.M.s offers a chance to improve the efficiency of conventional antimicrobials regimens and overcome the challenge of biofilm formation and other mechanisms of antimicrobial resistance [36]. Nanoparticles as vehicles for drugs to battle disease-causing pathogens are associated with their unique properties, which give them several advantages such as ease of Synthesis, improved solubility, extended antibiotic half-life, tissue targeting, and biocompatibility [36, 74].

The application of N.P.s in drug delivery has been demonstrated in several studies. For example, the targeted, effective, and safe treatment of pulmonary infection caused by *P. aeruginosa*, ROS-responsive nanoparticles, has been developed to capture moxifloxacin (MXF) [75].

Other studies have demonstrated that by using a rat central venous catheter (CVC) model of infection, sustained nitric oxide-releasing nanoparticles (NO-np) interfere with and prevent methicillin resistance, *S. aureus* adhesion, and biofilm formation [76].

Despite the reported development of bacterial resistance against gram-negative bacteria after repetitive usage of chemically synthesized AgNPs [77], conjugating them to clinically approved drugs has shown improved bactericidal activity. A study by Msari *et al*. proved an enhancement in antibacterial effectiveness of Cephradine (antibiotic) and Vildagliptin (antidiabetic) drugs following their conjugation to the AgNPs [78].

Application of antimicrobial nanomaterials in Vaccines

To protect humans against microbial infection, priming the immune system of the host so as to identify and target microbes has been certified as quite effective [70]. Active immunization against microbes relies mainly on the use of live mitigated organisms, lifeless organisms, or deactivated toxoids [79]. Once the pathogens overwhelm the physical obstructions of the host, they may possibly be identified by the innate immune system via pathogen-associated molecular patterns [80]. Live attenuated (LAV), inactivated vaccines, and toxoids are, however, associated with some disadvantages such as risks of virus transfer or return to the pathogenic form among others. Toxoid-derived vaccines like I.V.s are weak and oftentimes require several doses for the achievement of proper immune response [79]. Recombinant DNA methods have also been used to advance various vaccines such as DNA vaccines, subunit vaccines, and conjugate vaccines by connecting the weak antigens to stronger immunogens such as proteins. However, these vaccines are often experienced low immunogenicity than traditional vaccines and encounter early degradation after exposure to a hostile environment [70, 81]. Subunit vaccines have low immunogenicity due to a lack of components like the pathogen-related molecular configurations that are expressed on a microbe surface and can activate the pattern recognition receptors. They are thus formulated with an adjuvant molecule to elicit a robust immune response [82].

Nanotechnology has been embraced to address the above and other challenges associated with conventional and DNA-derived vaccines. Their small particle size facilitates their uptake by phagocytic cells, and mucosa-related lymphoid tissues, making nano-vaccines offer a prospect of enhancing humoral and cellular immune reactions due to efficient antigen presentation [83]. The antigen can be captured into the central part of the nanoparticle, while a specific and selective immune response is achieved by altering the surfaces of nano-carriers that allow the selective delivery of the antigens to the cell receptors [81, 83].

Owing to the sub-optimal vaccination procedures such as intravenous (IV) administered by parenteral injections, most current influenza vaccines do not develop robust immunity at lung mucosae. Reports have indicated that including AgNPs in the IV flu vaccine can results in diminished viral loads thereby preventing extreme lung inflammation following influenza contamination in mouse models [84]. Compared with commercial adjuvants such as silver salt, AgNPs have been reported to stimulate a robust antigen-specific IgA production with decreased toxicity.

Liposome nanoparticle-based subunit flu vaccine synthesized by encapsulating ten well-preserved B and T cell epitope peptides were found to induce a better protective immune response against a zoonotic swine influenza A infection using a pig model [85]. Dendrimer-conjugated vaccine platform for preventing Chlamydia trachomatis genital infection in the mouse model has also been reported [86].

Conclusion and Future perspective

Engineered N.M.s have revealed a substantial role in regulating microbial progression. They display bactericidal and bacteriostatic properties over several biochemical pathways that can be further explored to control microbial progression. Due to their unique properties, antibacterial N.M.s have been exploited in various ways, such as in the diagnosis, treatments of pathogenic infections, and drug delivery. Although great progress has been made in their applications, many challenges in their stability, toxicity, broad-based accumulation in the complexity of the biological system-based inflammation, false positives, remain to be solved. These challenges should therefore be addressed for their approval in clinical applications [87, 88]. Though these N.M.s have shown the potential in dealing with the increasing global challenge of antimicrobial resistance, an all-inclusive understanding of their mode of action and selection of the best N.M. for impending clinical translation remains a big challenge due to differences in their synthesis and testing methods. This calls for the development of more robust, standardized, in vitro assessment procedures that can be used to evaluate the antibacterial potency and effectiveness of these N.M.s.

More research is needed to synthesize N.M.s with controlled Physico-chemical properties to counter the mentioned challenges to ascertain the vulnerability of cytotoxicity, genotoxicity, and inflammatory response to human cells

upon N.M.'s exposure [88]. Also, more studies should focus on the surface modification of these N.M.s with biocompatible moieties such as polysaccharides or integration of specific recognition agents such as precise antibiotics or antibodies to treat identified pathogenic bacteria [89]. Finally, a fundamental understanding of the mode of action of the N.M.s when they interact with bacterial pathogens is important if they are to be used in clinical applications.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this review.

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Guidelines for Contributors

AIMS AND SCOPE OF THE PHARMACEUTICAL JOURNAL OF KENYA

The Pharmaceutical Journal of Kenya (PJK) is devoted to publishing original research manuscripts, reviews, letters to the Editor, and short communications. The PJK covers all aspects of medicines, health and life sciences. PJK provides a platform to all practitioners, researchers, academicians, students, and industrialists to share their ideas, knowledge, information and research findings among the people of their fraternity.

All submissions must be made in English.

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References cited only in tables or in legends to figures should be numbered in accordance with a sequence established by the first identification in the text of the particular table or illustration. Figures must be labelled at the bottom, whilst tables shall be labelled at the top.

The number of references should normally be restricted to a maximum of 25 for a full paper, whereby not more than 20% should be not more than 5 years old, and no more than 10% should be more than 10 years old. References older than 10 years should ideally be classical subject material references.

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The editors reserve the right to reject a manuscript for publication if it does not meet the requirements of the Pharmaceutical Journal of Kenya.

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