# Knowledge, perception and acceptability of microbicides among healthcare workers in Lagos, Nigeria

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Increasingly, women are seen to be affected by human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS), and now represent 43% of those infected globally and more than 55% of those infected in sub-Saharan Africa.[1] This increase can be attributed to physical vulnerability, susceptibility to rape, forced marriage, female trafficking, economic dependence and coercion.[2] This has led researchers to look at microbicides as a preventative option for women.

In addition, in view of some of the major drawbacks associated with condom use as a barrier to infection,[3] the introduction of microbicides is advantageous, especially in societies where women have little power. Clearly, use of microbicides would give women the option of taking action themselves, perhaps without the knowledge of their partner.[3]

Introduction of a microbicide could have a major public health impact. A microbicide with 60% efficacy, introduced into 73 low-income countries could avert 2.5 million HIV infections over three years in women, men and infants.[4]

Microbicides are anti-infective substances that can be applied topically before sexual intercourse to prevent the transmission of HIV and other sexually transmitted infections, and can prevent male-to-female and female-to-male HIV transmission.[5] Such substances are formulated as gels, creams, foams, impregnated sponges, films and suppositories.[5] They are not intended to replace other preventive measures, but give people a wider choice of potentially life saving methods of protection.

Presently, the United States Agency for International Development (USAID) is engaged in early clinical trial studies (phases I and II) of several potential microbicides (e.g., cellulose sulphate, polystyrene sulfonate, acid foam and PRO2000) that use different modes of action,[1] while phase III trials of other products are underway in many parts of the world, including south-west Nigeria.

Microbicides inactivate viruses present in the vagina, preventing them from attaching to or fusing with cellular targets and accumulating in the cells. In addition, microbicides can block viral replication in an infection.[6]

This study aims to assess the knowledge, attitudes and perceptions about microbicides among healthcare workers and examines their willingness to recommend microbicide use.

The study population comprised healthcare workers in hospitals and other health institution in Lagos, Nigeria, between October 2003 and February 2004. A total of 240 respondents (144 [60%] women, 96 [40%] men) were interviewed using structured questionnaires. Data were analysed using the Epi-Info 6.04 software.

The ages of the respondents ranged from 20 to 50 years (average age: 35 years; median age: 36 years) and comprised Christians (81.3%) Muslims (18.3%) and a small number with other religious affiliations (0.4%). Most (55.3%) were married, 39.1% were single, and the remainder (5.6%) were either divorced, separated or widowed. Breakdown of professional backgrounds was as follows: clinicians (26.3%), medical laboratory scientists (20.8%), nurses (17.5%), pharmacists (15.4%), researchers (11.3%) and other healthcare workers (8.7%).

Of the 240 respondents interviewed, 178 (71.7%) had heard of microbicides, while 68 (28.3%) had no knowledge of the product. Sources of information on microbicides among the respondents include conferences (34.5%), colleagues (27.6%), pamphlets (12.6%) and other sources (10.3%). With regard to the use of microbicides, 86% respondents were willing to use or recommend microbicides.

Correlation between knowledge and willingness to use microbicides showed a significant association between previous knowledge and a willingness to use and recommend microbicides ($P<0.05$). Furthermore, perception of the efficacy of microbicides against STIs/HIV showed that 12% thought them effective, 17% thought them ineffective and 71% were undecided.

The level of knowledge about microbicides among the respondents cannot be over emphasised considering their professional background. This is pertinent as this category of persons constitutes an important segment of stakeholders through which the product will be promoted and distributed for use in the long term. Hence, the high knowledge and acceptability of the product by the majority of the respondents is encouraging; however, efforts need be intensified to build on this positive trend.

Although the views expressed by most of the respondents at this stage are hypothetical, actual willingness to use the product will be determined when it is available for use and ascertained to be effective. The large number of the respondents who were undecided about the effectiveness of microbicides in the prevention of HIV/STIs could be attributed to knowledge of the previous failure of a similar product, nonoxynol-9 (N-9).[7]

Clearly, more effort is needed to educate the general population about the potential of microbicides. This is important because it is especially difficult for women to follow recommendations for HIV prevention. In sub-Saharan Africa, many women have little control over a partner's behaviour, and will be unaware of their infection status. Correct and consistent use of condoms has been shown to prevent the sexual transmission of HIV, but women are often unable to negotiate the use of condoms with their partners, hence the need for microbicides.

In view of fact that 70% of respondents preferred to use microbicides as a cream, with 60% willing to buy it in pharmaceutical stores and a few others preferring government clinics, it would seem that measures need to be put in place to prevent the circulation of fake and adulterated microbicides, particularly through uncertified pharmaceutical/chemist shops.

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# Bacterial contaminants in cosmetic products from a patient with cystic fibrosis

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A small bacteriological study is performed on several personal cosmetic items, which had been in current and regular use by a young adult with cystic fibrosis (CF) who had a history of chronic *Pseudomonas aeruginosa* chest infection and a recent history of *Burkholderia cepacia* genomovar IIIa chest infection. The aim of this small study is to determine the bacteriological diversity of the cosmetics, with particular reference to these two Gram-negative pathogens, and to assess the significance of these in terms of infection control issues for the patient and other patients in the CF unit.

Personal cosmetic products ($n$=24) that had been in regular and current use were obtained from an 20-year-old lady with a history of CF who had a previous history of chronic pulmonary infection with *P. aeruginosa* and a more recent history of infection with *B. cepacia* genomovar IIIa. The cosmetic products included the following items: blusher, brushes ($n$=4), concealer, glitter, highlighter, illuminator, liquid latex, lip gloss, sponge, make-up ($n$=8), make-up pencil, mascara ($n$=2) and theatrical blood.

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Bacteriological analysis was performed on each item by sampling using one of three methods. For brushes, the item was brushed (x5) against the shaft of a sterile swab and above a standard plate of *B. cepacia*-selective agar (BCSA; Mast Diagnostics, Bootle, Wirral, Merseyside, UK). For solid make-up, a standardised swabbing technique was used, involving premoistening of the swab with tryptone soya broth (TSB; Oxoid CM0129, Oxoid, Basingstoke, England) before aggressively swabbing the surface of the cosmetic product. For cosmetic material, aseptic removal of approximately 0.5 g material into 9 mL TSB was performed.

All swabs were inoculated directly on the surface of BCSA medium and were then enriched non-selectively in TSB (9 mL) at 37°C for 48 h. BCSA medium was incubated at 37°C for 48 h and then at ambient temperature on the bench for up to five days. All TSB enrichment broths were incubated at 37°C for 24 h, and then 20 mL was streaked individually on both BCSA and Columbia blood agar (Oxoid CM331) containing 5% (v/v) defibrinated horse blood, and incubated at 37°C for 48 h. Resulting colonies were purified on CBA and then identified using molecular methods, as described previously by Xu *et al*.[1]

None of the cosmetic items were positive for *B. cepacia* or *P. aeruginosa* and no culturable bacteria were detected in 21/24 (87.5%) of the items. Four cosmetic items were positive for Gram-positive organisms, as detailed in Table 1.

*P. aeruginosa* is the most important bacterial pathogen in patients with CF,[2] as demonstrated by high-prevalence data in most of the national CF registries. Chronic *P. aeruginosa* colonisation of the major airways, which leads to debilitating exacerbations of pulmonary infection, is the major cause of morbidity and mortality in patients with CF; hence, it is important to be able to detect *P. aeruginosa* from patients' sputum.

Recently, Emerson *et al*.[3] published their findings of a US Cystic Fibrosis Foundation (CFF) registry-based study, which showed that infection related to *P. aeruginosa* is a major predictor of morbidity and mortality. The eight-year risk of death parameter is 2.6 times higher in patients who had positive sputum cultures for this organism, and they have a significantly lower percentage predicted forced expiratory volume (FEV1). These workers suggested that early interventions may help decrease associated morbidity and mortality of young patients with CF.

More recently, Rosenfeld e*t al*.[4] described the patho-physiology and risk factors for early *P. aeruginosa* infection in

**Table 1.** Molecular identification employing 16S rDNA PCR and automated sequencing of four bacterial isolates from a variety of cosmetic products used by a patient with cystic fibrosis.

| Cosmetic item | Identification of contaminating bacterial organism |
|---|---|
| Highlighter | *Corynebacterium tuberculostearicum* |
| Eye pencil shavings | *Staphylococcus epidermidis* |
| Blusher | *Bacillus* sp. |
| Facial sponge | *Bacillus* sp. |

CF. They suggest that chronic lower airway infection with *P. aeruginosa* is associated with significant morbidity and mortality among CF patients. However, they also suggest that first acquisition of *P. aeruginosa* does not appear to cause an immediate and rapid decline in lung function, as early isolates are generally non-mucoid, antibiotic-sensitive and present at low densities. This suggests a possible window of opportunity for early intervention.

To date, there have been no reports on the bacterial composition of cosmetic products. Furthermore, the survival dynamics of problem Gram-negative pathogens in cosmetics are not known. Thus, further work is required to determine the survival and persistence of Gram-negative pathogens, including *P. aeruginosa* and *B. cepacia*, in these matrices. Until such studies report, it is recommended that patients with CF avoid sharing their cosmetic products among each other, as a precautionary measure, to help eliminate the potential for cross-infection with these pathogens.

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# Potentially misleading Western blot results in Lyme disease diagnosis


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The laboratory diagnosis of Lyme disease is complex[1] and serology remains the technique of choice. Recommended practice is a two-step process involving a sensitive screening enzyme immunoassay (EIA) followed by a more specific confirmatory Western blot for all EIA-positive and equivocal samples and for EIA-negative samples with a high clinical suspicion of Lyme disease (e.g., tick bite and erythema migrans).[1,2] However, Western blot results require careful interpretation.

The National Lyme Disease Testing Service Laboratory, Raigmore Hospital, Inverness, tests over 3000 samples annually from across Scotland, many of which are from complex clinical cases. It was noted recently that serum from a patient with confirmed parvovirus B19 infection cross-reacted with the in-house *Borrelia burgdorferi* IgG Western blot. This could lead to the wrong interpretation of Western blot results. This study aims to discover if other viral infections produce similar results with the *B. burgdorferi* IgG Western blot.


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The study group consisted of six patients found to contain IgM antibodies to parvovirus B19 infection (parvovirus B19 IgM EIA, Biotrin International), six with cytomegalovirus (CMV) IgM (CMV IgM EIA, Microgen Bioproducts) and six with Epstein-Barr virus (EBV) IgM (EBV VCA IgM EIA, Diasorin). All patients had good clinical evidence of viral infection (Table 1).

The 18 serum samples were analysed by commercial *B. burgdorferi* IgG/IgM EIA (Zeus Scientific, New Jersey, USA) and an in-house *B. burgdorferi* IgG Western blot. The EIA was performed according to the manufacturer's instructions and the Western blot was performed as described previously.[2]

The blots used contained sodium dodecyl sulphate-extracted *B. burgdorferi* (i.e., antigen). A commercial positive control (Zeus Scientific) and in-house positive control, with defined band patterns, were included in each blot run. The number, intensity and molecular weight of bands observed with each sample were recorded, and a negative, equivocal, weak positive or positive result assigned according to predetermined criteria.[3] The Western blot was repeated for all samples.

Previous or subsequent serum samples from five patients (serum samples 5, 8. 9, 11 and 12 [Table 1]) who reacted with the *B. burgdorferi* IgG Western blot were also tested by the *B. burgdorferi* IgG Western blot to determine *B. burgdorferi* status.

All samples tested were EIA negative (Table 1). However, as the infections studied may produce clinical symptoms similar to Lyme disease (such as a rash), a Western blot may be performed on EIA-negative samples, based on clinical suspicion.[2,3] Western blot may also be performed on EIA-negative samples if they have been referred from another laboratory for confirmation.

Some of the polypeptides from a *B. burgdorferi* extract may react with patient antibodies that are not specific to *B. burgdorferi*.[4] These include the 41 kDa flagellin polypeptide, which, although it must be detected for a positive diagnosis, is known to cross-react with antibodies to proteins from other bacteria.[4,5] Other polypeptides in this category include those of 44, 56, 62, 64, 72 and 82 kDa. In contrast, bands with 18, 22, 32, 34, 39, 46, 58 and 92 kDa polypeptides are generally accepted as specific for *B. burgdorferi*.[1,6]

Three of six parvovirus B19 IgM-positive serum samples reacted with specific *B. burgdorferi* antigens and the 41-kDa antigen (Table 1). The antibody response to parvovirus B19 is directed mainly against the two structural proteins of the viral capsid (VP1 [83 kDa] and VP2 [58 kDa]).[7,8] Although the molecular weights of these polypeptides are different from the *B. burgdorferi*-specific bands detected here (Table 1), they may share sufficient homology at particular epitopes for parvovirus B19-specific antibody to bind with *B. burgdorferi*-specific polypeptides.

Five of six CMV-IgM positive sera produced equivocal or

**Table 1.** *Borrelia burgdorferi* IgG/IgM EIA and IgG Western blot results on serum samples from patients with good evidence of parvovirus B19 ($n=6$), CMV ($n=6$) and EBV ($n=6$) infection.

| Organism | Serum | Clinical information | Western blot | | |
|---|---|---|---|---|---|
| | | | 41 kDa band | Specific bands | Result |
| Parvovirus B19 | 1 | Rash on body, muscle aches | Yes | 0 | Negative |
| | 2 | Arthropathy, abnormal LFT's | Yes | 92 | Equivocal |
| | 3 | Viral symptoms, reactive arthropathy | Yes | 0 | Negative |
| | 4 | Viral infection with arthralgia | Yes | 0 | Negative |
| | 5 | Generalised macular, confluent rash for 24 h | Yes | 34 kDa | Equivocal |
| | 6 | Inflammatory arthritis | Yes | 32 kDa | Equivocal |
| CMV | 7 | Pyrexia ?neutropenia | Yes | 0 | Negative |
| | 8 | ?CMV, epigastric discomfort | Yes | 34 kDa | Equivocal |
| | 9 | Unwell for past month, malaise | Yes | 39 kDa | Equivocal |
| | 10 | Viral illness, increased ALT, low WCC | Yes | 34 kDa | Equivocal |
| | 11 | Pneumonitis, respiratory failure, pericardial and pleural effusions | Yes | 58, 92 kDa | Wk positive |
| | 12 | Sore throat, recurrent glandular swelling, TATT | Yes | 92 kDa | Equivocal |
| EBV | 13 | ?Glandular fever | Yes | 0 | Negative |
| | 14 | Previous CFS. Persistently EBV+, now asymptomatic | Yes | 0 | Negative |
| | 15 | TATT. Previous infectious mononucleosis | Yes | 0 | Negative |
| | 16 | Recent glandular fever | No | 0 | Negative |
| | 17 | Ongoing pharyngitis | Yes | 0 | Negative |
| | 18 | Cervical lymphadenopathy | Yes | 0 | Negative |

weak positive *B. burgdorferi* Western blot results (Table 1). The individual structural proteins of CMV recognised by sera from IgG- and IgM-positive patients are 155, 149, 82.5, 74.5, 67, 57, 55, 38.5 and 28 kDa polypeptides.[9] The 38.5 and 57 kDa polypeptides are of similar size to the 39 and 58 kDa polypeptides of *B. burgdorferi*, but there is no information about their homology. Other shared epitopes are suggested as the CMV IgM-positive sera also detect 34 and 92 kDa polypeptides on the *B. burgdorferi* blot.

In one case (sample 11, Table 1) the result was a weak positive. Although concurrent *B. burgdorferi* infection cannot be ruled out in the patients with parvovirus B19 and CMV infection whose sera cross-reacted, the *B. burgdorferi* IgG Western blot results from available previous or follow-up sera (samples 5, 8, 9, 11 and 12, Table 1) suggest that none of the patients had current or previous *B. burgdorferi* infection.

None of the EBV IgM-positive serum samples were equivocal or positive by *B. burgdorferi* IgG Western blot. This was surprising as there is excessive production of antibodies during EBV infection and cross-reactions are observed frequently in tests for other infections. However, it confirms the lack of cross-reactions observed when Engstrom *et al*. tested sera from EBV patients with their *B. burgdorferi* IgG Western blot.[10]

The findings presented here highlight the problems with a Western blot result. Western blot needs to be sensitive to avoid false-negative results, particularly in early Lyme disease. Most cross-reactions in this study produced equivocal results, which, although inconclusive, are beneficial as repeat samples are requested automatically in order to help confirm or refute the presence of infection. A repeat equivocal result means that the clinical picture would have to be closely considered. The weak positive result obtained with CMV was more problematic. Knowledge of these cross-reactions enables us to better advise our users, and emphasises the need for good clinical information in the interpretation of laboratory results.

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# Diagnosis of Helicobacter pylori infection among patients with dental caries by stool antigen test

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*Helicobacter pylori* is the causative agent of gastritis, peptic ulcer disease and a risk factor in the development of gastric cancer.[1] The route of transmission of *H. pylori* is currently under debate, although evidence suggests that it is predominantly by direct person-to-person contact. Transmission routes vary between the developed world and developing countries. In developed countries, transmission is largely by the oral-oral route, whereas in developing countries it is by the faecal-oral route. It is also suggested that *H. pylori* exists in the natural environment.[2]

As the oral cavity is a possible reservoir for the organism, it provides a possible tool for the rapid and non-invasive diagnosis of infection. Current studies indicate that *H. pylori* is present in dental plaque, although low numbers have been reported in individual samples and numbers vary between sites in the mouth.[3]

The presence of the organisms in plaque may be intermittent, perhaps as a result of gastro-oesophageal reflux.[3,4] In addition, there is some controversy about whether or not dental plaque is a significant source for re-infection of the gastric mucosa in patients with fair to poor oral hygiene.[3]

Nasrolahei *et al*.[5] showed no significant association between *H. pylori* colonisation in dental plaque and gastric infection. Matsuda and Morizane[6] screened for the risk of acquiring *H. pylori* infection among dental professionals and non-dental professionals, and showed that the former group was at greater risk of infection. Al-Hawajri *et al*.[7] concluded that dental plaque may be a candidate reservoir for *H. pylori*, that medical equipment may contribute to *H. pylori* transmission and that sample collection techniques can bias the true prevalence of *H. pylori* in a population.

During recent years, non-invasive methods to detect *H. pylori* infection have gained importance. Current guidelines that recommend *H. pylori* eradication treatment without performing endoscopy in certain patients highlights the importance of non-invasive tests.

The stool antigen test allows sensitive and specific non-invasive detection of *H. pylori*, is cost effective and has been used in both the diagnosis of infection and to confirm *H. pylori* eradication after treatment. In Nigeria, where loss of power is frequent and *H. pylori* culture is difficult, such a test would help in the treatment and eradication of *H. pylori*.

This study aims to detect *H. pylori* in dental plaque and in gastric biopsies from patients with a range of dental problems, and to correlate results with a stool antigen test.

Forty-one patients (age range: 4–55 years, mean: 30.9 years) presenting with a range of dental problems had stool samples screened for *H. pylori* infection using a stool antigen test (Dako). Gastric biopsies were taken after the patients gave informed consent. All the patients had not been on any medication. Biopsy samples were also screened for *H. pylori* using the CLO test and culture. Dental plaque was screened for rapid urease production using the CLO test and was also cultured for *H. pylori*.

Plaque samples obtained from teeth cavities were placed in sterile bottles containing tryptone soya broth for culture and also directly inoculated into a CLO test kit for detection of rapid urease production.

Two biopsy samples were obtained from each patient: one was cultured using Dent's medium in a candle extinction jar at 37°C for three to 10 days, while the second was added to CLO test medium to screen for rapid urease production.

For the stool antigen test, an enzyme-linked immunosorbent assay (ELISA) method using monoclonal antibodies for direct, non-invasive detection of *H. pylori* was employed. Briefly, the supernatant of a faecal suspension was added to the wells of the ELISA microplate, together with horseradish peroxidase (HRP)-labelled anti-*H. pylori* monoclonal antibody. Following incubation and subsequent washing, enzyme substrate (tetramethylbenzidine [TMB]) was added to each well. In this assay, HRP oxidised TMB to a blue coloured product. Addition of a stop solution produced a colour change to yellow and the intensity was measured spectrophotometrically.

Positive and negative controls were included with each test run. The positive control at an absorbance of 450/620-650 nm ($A_{450/620 \text{ to } 650}$) was >1.00 ($A_{450}$ >1.04), while the negative control at $A_{450/620\text{-}650}$ was <0.10 ($A_{450}$ <0.14). Test results were interpreted as follows: specimens with *A* values ≥0.15 were regarded as positive for *H. pylori* antigen, while specimens with *A* values <0.15 were regarded as negative for *H. pylori* antigen.

Patients were defined as infected when positive results were obtained with the stool antigen test or culture, or when a positive CLO test was obtained on dental plaque.

Fourteen (34%) patients had peptic ulcer disease, while 27 (66%) had marginal gingivitis and were either normal or had mild gastritis. Irrespective of disease status, all patients were found to have *H. pylori* by the stool antigen test (13 [31.7%] males, 28 [68.3%] females). Culture of dental plaque detected *H. pylori* in only 5% of patients, while the CLO test was positive in 56% of cases. Culture of gastric biopsy samples showed a 10% isolation rate, while the CLO test was positive in 61% of case.

A variety of highly sensitive and specific detection methods have been evaluated for the detection of *H. pylori* infection. Invasive tests are usually associated with problems

of cost, especially in developing countries. There are several non-invasive methods (e.g., [$^{14}$C] urea breathe test [UBT] and serology) but UBT alone is very expensive and serological tests do not measure active infection accurately. Thus, it is imperative to find an easy, cheap and accurate non-invasive test for diagnosing *H. pylori* infection. Various studies have examined the accuracy of the stool antigen test, a non-invasive test for the diagnosis of *H. pylori*.[8–10]

The stool antigen test has long been known as a useful diagnostic method for the detection of *H. pylori*. The present study is the first of its kind in Nigeria and results show that all patients in the study who reported with various dental problems were positive for *H. pylori* infection by the stool antigen test. The presence of the organisms in plaque obtained from asymptomatic individuals might have been due to gastro-oesophageal reflux, and might serve as a source of infection or re-infection.

The rapid urease test (CLO test) showed that *H. pylori* was present in approximately 60% of individuals who reported with dental problems alone. Although culture of *H. pylori* from dental plaque and biopsy was relatively low (5% and 10%, respectively), it demonstrates the problems associated with mismanagement of the disease when culture alone is relied on in a developing environment.

A previous report from Nigeria, where *H. pylori* isolation rate was 27% by culture, corroborates this view.[11] Prevalence by serology is shown to be 85%, while by Gram stain it is 58%.[12] However, following the test-and-treat guidelines adopted in countries where *H. pylori* prevalence is >20%, the stool antigen test would be a better option in Nigeria, as it is affordable and a more reliable means of diagnosing *H. pylori* infection in asymptomatic individuals.

The results of the present study demonstrate the significance and affordability of the stool antigen test as a diagnostic tool in the absence of culture; thus, the stool antigen test would appear to be a better option for the non-invasive detection of *H. pylori* in Nigeria. Currently, further studies are underway to confirm the usefulness of the stool antigen test in the diagnosis and also the eradication of *H. pylori* after treatment.

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