**Supplementary Table S3. Overview of cross-sectional studies assessing bacterial contamination of antiseptics and disinfectants used in healthcare facilities in low- and middle-income countries.** Country income level is based on the data from the World Bank (World Bank2022) at the year of publication of the article. Product names are listed as mentioned in the referred articles; in case of brand names or jargon, the generic name is written in brackets and italics. The product categories (antiseptics/disinfectants) are listed according to the categories mentioned in the referred articles. Bacterial names are listed as mentioned in the referred articles, names according to actual nomenclature (*List of Prokaryotic names with Standing in Nomenclature* and updated species names were used [33]) are written between brackets and used in the "Summary/Comment". When written in brackets; the term "*modified Kelsey-and-Maurer*" was not used in the original articles but has been added for all articles using a dilution of the liquid product in nutrient broth and subsequent plating on solid culture media. Abbreviations: AST = antibiotic susceptibility testing, CHG = chlorhexidine gluconate, CHG-QUAT = quaternary ammonium compound, CFU/ml = colony forming units/ml, ID = Identification, LIC = low-income country, MIC = middle income country, PCMX = chloroxylenol, QUAT = quaternary ammonium compound.

| **Author – Year**<br>**Country (income level)**<br>**Setting** | **Study design, products assessed, findings** | **Methods**<br>**Risk factors (assumed or observed)** |
|---|---|---|
| Khor 1977<br>Malaysia (MIC)<br>No detail on the hospitals (n = 4) | Objective: to assess the contamination of products and resistance to disinfectants<br>Design: Cross-sectional survey of disinfectants from discard jars (n = 67)<br>Products: disinfectants<br>Dettol (*PCMX*) : dilution 1/10 to 1/20 (no stock concentration mentioned) (n = 26)<br>Hibitane (*CHG*) : dilution 1/200 to 1/2000 (no stock concentration mentioned) (n = 36)<br>Cetavlon (*QUAT*): no concentration mentioned (n = 2)<br>Milton (*chlorine*): no concentration mentioned (n = 2)<br>Contamination ratio and contaminating flora: overall 16/67 (24%) products contaminated<br>Jars filled with Dettol: 11/26 (42%) *Moraxella* spp., *Pseudomonas* spp. and *Alcaligenes* spp.<br>Jars filled with Hibitane: 4/36 (11%) *Moraxella* spp., *Pseudomonas* spp., *Flavobacterium* spp.<br>Colony count: 12/67 (18%) higher than $10^5$ CFU/ml | Methods<br>Sample selection not described<br>Kelsey-and-Maurer method, incubation at 37°C for 48 h<br>Method for colony count not specified<br>Neutralizer: Tween 80 (3%)<br>ID: Conventional phenotypical methods<br>No AST done<br>Risk factors<br>Assumed:<br>Inaccurate concentration<br>Infrequent changing of the solutions<br>Topping-up<br>Discard jars without cleaning<br>Presence of organic materials<br>and inappropriate choice of disinfectant |
| Olayemi 1994<br>Nigeria (LIC)<br>Tertiary hospital Urban medical centers (n = 3) | Objective: to assess the contamination of products<br>"to appraise sterility of working dilutions of common disinfectants"<br>Design: Cross-sectional survey of disinfectants working dilutions (n = 18)<br>Products: disinfectants<br>Dettol (*PCMX*) Hibitane (*diguanide*) (CHG)<br>Trichlorophenol-TCP (*phenol*) Savlon (*CHG-QUAT*)<br>Methylated spirit (*denatured ethanol*) | Methods<br>Sample selection not described<br>Kelsey-and-Maurer method, no details given<br>Neutralizer: not mentioned<br>ID: conventional phenotypical methods<br>No AST done |

| | | |
|---|---|---|
| | Contamination ratio and contaminating flora : 5/18 (27.8%) samples<br>Eusol (*chlorine*) : *Pseudomonas aeruginosa*, *Bacillus* spp., *Staphylococcus aureus*<br>Potassium iodide : *Pseudomonas aeruginosa*, *Bacillus* spp.<br>Methylated spirit*: Pseudomonas aeruginosa*, *Bacillus* spp.<br>Potassium permanganate: *Escherichia coli*<br>Tincture benzoate: *Pseudomonas* spp. | Risk factors<br>No information about Risk factors |
| Danchaivijitr 1995<br>Thailand (MIC)<br>Tertiary hospital | Objective: to assess the contamination of products<br>Design: Cross-sectional survey of hospital antiseptics: 23 wards, 141 samples, both pharmacy and ward stock and in-use products.<br>Products: antiseptics<br>Acriflavine* / Mercurochrome*<br>Ethanol / Iodophor<br>Hydrogen peroxide / Iodine tincture<br>Methimasol* / CHG-Cetrimide (*CHG-QUAT*)<br>Contamination ratio and contaminating flora:<br>Mercurochrome: 28/44 (63.3%) of in-use vials, 3/3 stock vials (pharmacy)<br>Contaminating flora: In-use vials: Gram-negative bacteria (n = 12, identification not specified), Gram-positive bacilli (n = 15) and micrococci (n = 1), Stock: Gram-positive bacilli<br>CHG-cetrimide: 6/41 (14.6%) in-use vials, no growth in 3 stock vials at the pharmacy<br>Contaminating flora: Gram-negative bacilli (n = 4, one *Acinetobacter anitratus*) and Gram-positive bacilli (n = 2)<br>No growth from alcohol-based products (n = 25), acriflavine (n = 7), hypochlorite (n = 4), iodophor (n = 18); iodine tincture (n = 18) and methimasol (n = 9)<br>Interventions – Recommendations<br>Hospital policy on the selection of antiseptics (Infection Prevention & Control Committee)<br>Monitoring of contamination of antiseptics | Methods<br>Selection of all antiseptic products<br>Use of neutralizer (type not mentioned)<br>Culture methods not specified<br>ID: conventional phenotypical methods<br>No AST done<br><br>Risk factors<br>Observed:<br>Working area not clean<br>Dilution with tap water<br>Non-disinfected containers |
| Keah 1995<br>Malaysia (MIC)<br>General hospitals<br>(n = 6) | Objective: to assess the contamination of products<br>Design: Cross-sectional surveys sampling freshly prepared samples at the pharmacy (5% of samples, working dilutions), ward stock samples and in-use samples<br>Products: disinfectants<br>Alcohol 70% / CHG water-based with 1% $NaNO_2$<br>Amphyl (*chlorophenol, phenol)* / *(note: $NaNO_2$ = preservative)*<br>Carbolic acid (*phenylic acid, phenol*) / Cidex 2% *(Glutaraldehyde)*<br>Cetrimide (QUAT) / Dettol *(PCMX)*<br>CHG in 70% alcohol / Lysol*<br>CHG (*water-based CHG*) / Povidone iodine | Methods<br>Random selection of 5% of freshly diluted disinfectants<br>*Modified Kelsey-and-Maurer method*: contamination defined at 5 colonies or 250 CFU/ml<br>Neutralizer (3% w/v Tween 80) added<br>No AST done |

Contamination ratios per hospital ranged from 0.5% to 19.5%, overall, 7.9%

Contamination ratios per category:

Pharmacy: 7.9% (342/4316 products): ranging from 0.5% to 19.5%

Per hospital: 0.5% - 0.6% - 1.4% - 6.0% - 15.8% - 19.5%

Contaminating flora (n = 354): *Pseudomonas* spp. 42.4%, *Moraxella* 22.0%, *Flavobacterium* 11.6%, *Enterobacter* 4.2%

Stock in ward: 16.1% (367/2278 products)

Contaminating flora (n = 214) *Pseudomonas* spp. 48.6%, *Moraxella* 17.8%, *Acinetobacter*, 8.9%, *Flavobacterium* 7.0%

In-use product (in ward): 16.4% (1519/9265 products)

Contaminating flora: *Pseudomonas* spp. 44.3%, *Bacillus* spp. 13.0%, *Enterobacter* 9.5%, *Acinetobacter* 7.3%

Contamination per product:

| | | **Pharmacy** | **Stock in Ward** | **In use** |
|---|---|---|---|---|
| Alcohol | 70%: | 14.7% | 2.3.% | 38.3% |
| Amphyl | 1/20 | ND | ND | 0.0% |
| | 1/40 | ND | ND | 0.0 |
| | 1/50: | 0.0% | 0.0% | 43.9% |
| | 1/100 | ND | ND | 38.5% |
| | 1/200 | 0.3% | 0.7% | 43.8% |
| | 1/2000 | ND | ND | 50.0 |
| Carbolic | 1/20 | 0.0% | 1.0% | 4.4% |

Risk factors: Assumed

Not following procedures of the Ministry of Health

Dilution

Reprocessing containers and caps

Prolonged use (>1 day for diluted products)

| | | | | |
|---|---|---|---|---|
| | 1/40 | ND | ND | 14.3% |
| Cetrimide | undiluted | ND | 0/2 samples | ND |
| | 1/100 | 7.3% | 9.9% | 5.3% |
| CHG in 70% alcohol | 1/200 | 0.9% | 3.3% | 4.8% |
| | 1/2000 | 0.0% | 0.0% | 12.5% |
| CHG water-based | 1/20 | 0.0% | no data | no data |
| | 1/5000: | 58.1% | 53.3% | 81.8% |
| | Note: CHG water-based at 1/1000 in-use: 50.7% | | | |
| CHG water-based 1% $NaNO_2$ | 1/2000 | ND | 0.0% | 21.4% |
| | 1/5000 | 19.1% | 25.0% | 21.8% |
| Cidex 2% | Undiluted | 0/1 samples | 0/1 samples | 6.3% |
| Dettol | 1/20 | 100% | 100% | |
| | 1/40 | 20% | 62.5% | |
| Hypochlorite | 1/10 | 6.7% | 0% | 7.3% |
| Lysol* | Undiluted | ND | 0/1 product | 0.0% |
| | 1/40 | 1.5% | 0.0% | 23.3% |
| Povidone iodine | Undiluted | no data | no data | 0.0% |

Contaminating flora per product:

Alcohol 70%: *Enterobacter* spp., *Pseudomonas* spp. (including *P. aeruginosa*), *Hafnia alvei*, staphylococci, others

Cetrimide: *Pseudomonas* spp. (including *P. aeruginosa*)

Chlorhexidine (no detail about water-based/alcohol/$NaNO_2$): *Pseudomonas* spp. (including *P. aeruginosa*) > *Acinetobacter* spp., *Alcaligenes* spp., *Bacillus* spp., *Enterobacter* spp., *Flavobacterium* spp., *Klebsiella* spp., *Moraxella* spp., *Hafnia alvei*, unknown, others.

Cidex: *Enterobacter* spp., *Bacillus* spp., *Pseudomonas* spp. (including *P. aeruginosa*), staphylococci, others

Hypochlorite: *Acinetobacter* spp., *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp. (including *P. aeruginosa*), unknown, others

Phenolics: *Pseudomonas* spp. (including *P. aeruginosa*) > *Bacillus* spp., *Acinetobacter* spp., *Alcaligenes* spp., *Enterobacter* spp., *Flavobacterium* spp., fungi, *Moraxella* spp., *Klebsiella* spp., unknown, others

---

Kajanahareutai 1995

Thailand (MIC)

Secondary hospital

Objective: to assess the contamination of products

Design: Cross-sectional study: in-use products (n = 244) in operating room, delivery room, and intensive care unit

Products: disinfectants

CHG – Cetrimide (*CHG-QUAT*) Phenol

QUAT (not specified) Hypochlorite (*chlorine*)

Methods

Sample selection not described (sampling twice a week)

Kelsey-and-Maurer method: neutralizer for CHG-cetrimide and QUAT (Tween 80 3% in nutrient broth), 1/10 dilution, inoculation

| | | |
|---|---|---|
| | Contamination ratio and contaminating flora per product:<br>CHG-Cetrimide dilution 1/100: 1.6% (2/126)<br>Contaminating flora: *Pseudomonas maltophilia (Stenotrophomonas maltophilia)* and *Achromobacter xylosoxidans*<br>QUAT: no contamination at dilutions 1/100 (40 samples) and 1/500 (6 samples)<br>Phenolic 2%: 2.9% (1/34): *Acinetobacter lwoffii*<br>Hypochlorite 0.05%: 12.5% (2/16): *Enterobacter cloacae*, *Enterobacte*r spp., *Acinetobacter calcoaceticus*, *Pseudomonas* spp.<br>Hypochlorite 1%: none of 12 samples contaminated | on nutrient agar, incubation at 35°C for 3 days and room temperature for 7 days<br>Contamination was defined at > 250 CFU/ml<br>ID: conventional phenotypical methods<br>No AST<br><br>Risk factors<br>Observed:<br>Miscalculation: hypochlorite 0.05% instead of 0.5% (prepared by ward staff) |
| Arjunwadkar 2001<br>India (LIC)<br>Tertiary hospital | Objective: to assess the contamination of products<br>Design: Cross-sectional study: freshly prepared, In-use samples (Day 5 of use, 4 hospital wards) and stock samples of undiluted products), comparison with nosocomial clinical isolates<br>Product: antiseptics<br>Benzalkonium chloride (QUAT)<br>Contamination ratios per product category and wards:<br>Freshly prepared samples: 14.8% (15/284) contaminated<br>In-use samples: 15.3% (28/183) contaminated<br>Stock bottles with undiluted samples: no contamination<br>Differences in contamination ratios between wards<br>Contaminating flora (n = 66 isolates)<br>Non-fermentative Gram-negative (45.5%), *P. aeruginosa* (30.2%), *Klebsiella* spp. (22.7%)<br>Comparison with clinical isolates from healthcare-associated (nosocomial) infections:<br>Urine from 15 symptomatic catheterized patients: 4/9 isolates matched with those grown from antiseptics (same ID and AST results) | Methods<br>Sample selection not described<br>Direct plating on blood agar<br><br>ID: conventional phenotypical methods<br>AST: disk diffusion, guidelines not mentioned<br>No detail on antibiotics tested nor AST profile<br><br>Risk factors<br>Assumed:<br>Inadequate boiling of containers<br>Improper handling |
| Ogunsola 2002<br>Nigeria (LIC)<br>Tertiary hospital | Objective: to assess the contamination of products and its contributing factors<br>Design: Cross-sectional survey assessing disinfectant in-use and stock solutions (pre-diluted, unopened), boiled and tap water used for dilution. Observations of preparation and storage.<br>Products: disinfectants<br>CHG 4% and 5% — CHG-Cetrimide (*CHG-QUAT*)<br>Hydrogen peroxide 6% with stabilizer — Hypochlorite 3.5% household (*chlorine*)<br>Contamination per category:<br>Stock samples 50.8% (33/65), In-use samples 63.1% (82/130) contamination<br>Contamination ratio per product: (undiluted/lowest dilution versus highest dilution) | Methods<br>Sample collection not described<br>Neutralizer used (Tween-80 broth)<br>*Modified Kelsey-and-Maurer method*, filtration of water samples<br>Incubation at 37°C and room temperature, 3 days<br>ID: conventional phenotypical methods<br>AST: disk diffusion, NCCLS 1993 but results not reported |

CHG 5% (Hibitane)
Stock: Undiluted: 0/2 – 1/200: 3/5 – 1/250: 3/5 – 1/500: 3/5 – 1/1000: 3/5 – 1/2000: 4/5
In-use: 1/200: 7/11 – 1/250: 8/10 – 1/500: 9/11 – 1/1000: 9/11 – 1/2000: 13/15
Isolates : *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Bacillus* spp., *Enterobacter* spp., *Klebsiella aerogenes*, staphylococci
CHG 4% (Hibiscrub)
Stock undiluted 5/12, In-use undiluted 9/20
Isolates : *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, staphylococci
CHG-Cetrimide (Savlon (CHG 0.3% - cetrimide 3%))
Stock undiluted 5/13; In-use undiluted 10/25
Isolates *: Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella aerogenes*, *Enterobacter* spp., staphylococci , *Bacillus* spp.
Hydrogen peroxide 6%
Stock undiluted 7/11; In-use undiluted 14/22
Isolates : *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Bacillus* spp., *Klebsiella aerogenes*
Hypochlorite
Stock undiluted (3.5%) 0/2, In-use (diluted 1/10): 3/5
Isolates : *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus* spp.

Contamination according to time of storage/use:
Contamination ratio increased according to storage time: For 130 samples: 1 – 24h: 36.8%; 24 – 48h: 65.9%; > 48h: 80.4%
Most products were heavily contaminated after 48 hours

Contaminating flora: (for details, see above)
Gram-negative bacteria: 91.0% of isolates; *P. aeruginosa* (67.2% of isolates) > *Proteus* mirabilis (16.4%), *Enterobacter* (6.6%), > *Klebsiella*, *E. coli* (6.9% each) > other isolates
Polymicrobial contamination in 15.2% of samples

Contamination per ward:
Contamination ratio and flora similar across wards
Contaminating flora similar to flora causing healthcare-associated infections, e.g., post-operative wound infections

Other findings:
Boiled tap water used for dilution grew with *P. aeruginosa* and coliforms
Swabs from inside stock bottles grew various organisms (no detail given)

Risk factors:
Observed
Tap water used for dilution contaminated
Duration of storage with deterioration of product
Containers were not always washed before refill

| | | |
|---|---|---|
| Gajadhar 2003<br>Trinidad and Tobago (MIC)<br>Four hospitals (no detail about hospital level) | Objective: to assess the contamination of products and the antimicrobial susceptibility of contaminating bacteria<br>Design: (i) Cross-sectional study, 4 hospitals; pharmacy stock, pre-use and in-use diluted products in pediatric/neonatal, and surgical wards; (ii) Questionnaire about types and use of antiseptics and disinfectants in 11 hospitals<br>Products: antiseptics/disinfectants<br>CHG (no concentration mentioned)<br>Savlon (CHG and cetrimonium bromide (*CHG-QAUT*, 1%)<br>Methylated spirit (denatured *alcohol*)<br>Contamination ratio: 11/180 (6.1%) in-use products, bacterial counts < 10 to 3.62 $10^4$ CFU/ml<br>Contamination ratio per product: CHG: 2/60 (3.3%), Savlon: 9/60 (15.0%), Alcohol: 0/60<br>Contaminated products per ward: 2/4 hospital had contaminated products; 6/11 contaminated products were diluted products in the pharmacy, 5/11 were pre-use and in-use products in the ward. No contamination was found in 36 stock products at the pharmacy.<br>Contaminating flora: *Pseudomonas* spp. | Methods<br>Sample selection not described<br>Kelsey-and-Maurer method: dilution of 1 ml of sample in 9 ml of nutrient broth, no neutralizer, inoculation of nutrient agar with 200 µl of 1/10 dilution in duplicate, incubation at 25°C for 72 h and 37°C for 24 h respectively<br>ID: conventional phenotypic methods<br>AST: disk diffusion, guidelines not mentioned, no full AST data displayed. Resistance ranged from 25% (tetracycline) to 100% (ceftriaxone and streptomycin), MDR not reported<br>Risk factors:<br>Questionnaire (11 hospitals):<br>Pharmacists (n = 7) or pharmacy attendants (n = 4) were in charge of preparation and distribution of products<br>Water used for dilution and washing of containers: tap water (n = 9), distilled water (n = 2), boiled water (n = 1)<br>Utensils for preparation/dilution were not sterilized in 9 hospitals<br>Containers used for distribution of products in the wards were recycled, non-sterile multi-use containers |
| Danchaivijitr 2005<br>Thailand (MIC)<br>39 Hospitals:<br>University (n = 2)<br>Regional (n = 4)<br>Provincial (n = 10)<br>District (n = 19)<br>Private (n = 4) | Objective: to assess the contamination of products and its contributing factors<br>Design: Large-scale study comprising a (i) Cross-sectional survey (39 hospitals, 16.142 samples) and (ii) a Questionnaire about preparation and use of antiseptics/disinfectants. Products were assessed freshly at preparation and in products at days 3, 5 and 7 of use/storage.<br>Products and Contamination ratio per product: antiseptics/disinfectants<br>Alcohol (ethanol 70%), n = 6562): 2.3%<br>Chlorhexidine 4% (n = 2722): 2.8%<br>Chlorhexidine 0.5% (n = 1054): 2.0%<br>Povidone iodine 7.5% (n = 3648): 0.5%<br>Povidone iodine 10% (n = 671): 0.0%<br>Tincture iodine 1-2% (n = 337) : 0.0%<br>Lysol* 2% (n = 872) : 4.7%*<br>Sodium hypochlorite 0.5% (n = 276): 1.0% (*chlorine*) | Methods<br>Hospitals (n = 39) were enrolled by stratified randomization to represent categories of hospitals and their geographical localization<br>Sample selection not described<br>Sample processing: (i) direct plating of 10 µl on blood agar (ii) (*modified Kelsey-and-Maurer method*): dilution of 1 ml sample in 9 ml of tryptic soy broth (no neutralizer), spread of 10 µl on a blood agar plate.<br>ID: conventional phenotypic methods, no AST |

| | | |
|---|---|---|
| | *High ratio ascribed to preparation in the ward (unskilled staff, non-sterile containers<br><br>Contamination ratio per category of hospitals:<br>Overall: 1.8% of samples, ranging from 0% (university hospitals) to 3.3% (provincial hospitals)<br>Highest ratios: 9.7% of CHG in district hospitals was contaminated; 25.0% of Lysol in reference hospitals was contaminated<br><br>Contaminating flora (n = 290 isolates):<br>Coagulase negative staphylococci: 35.9%<br>*Bacillus* spp.: 20.0%<br>*P. aeruginosa:*18.3%<br>*Enterobacter* spp: 13.8%<br>Other: 7.9%<br><br>Contamination ratio according to time of use:<br>Contamination ratios at days 1, 3 and 7 after preparation: 0.7%, 3.7% and 7.0% respectively | Risk factors:<br>Observed<br>Unskilled personnel preparing products in the ward<br>Preparation places (not suitable for aseptic preparation)<br>Use of unsterile containers, topping-up<br>No expiry date of diluted products, prolonged use (> 7 days)<br><br>Questionnaire:<br>Products were prepared in different places and by different staff: pharmacy, (unskilled) nurses in the wards, central sterile supply department or an external company<br>Less than 50% of containers was sterilized (dry heat or autoclave)<br><br>A total of 26.4% of samples were in use at Day 7 after preparation and in 17.8% products, there was no specified expiry date |
| Tytler2006<br><br>Nigeria (LIC)<br><br>8 University, 3 public and 5 private health facilities | Objective: to assess the contamination of products and its contributing factors<br><br>Design: Multicenter study assessing diluted and stock samples (n = 104)<br><br>Products: disinfectants<br>Tiscol Germicide (chlorinated *phenol* 35% w/v)<br>Tiscol (*PCMX 1.2% w/v*)<br>Morigad (Dichloro Meta Xylenol 2.5% DCMX, *Phenol*)<br>Eusol (*chlorine* – no concentration provided)<br>Z-Germicide (*Phenol:* 7% tar acid phenol and 2% cresol)<br>Purit (*CHG-QUAT :* CHG Bp 0.3% - Cetrimide Bp 3.0% *w/v)*<br>Harpic* *(cleaning product)*<br>Dettol (*PCMX*)<br>Hibiscrub (*CHG*)<br>Savlon (*CHG–QUAT*) | Methods<br><br>Sample selection not described<br>Viable counts by pour plate method (phenol-based) and 0.45 µm membrane filtration (CHG and cetrimide); duplicate inoculation<br><br>ID: conventional phenotypic methods<br><br>No AST |

| | | |
|---|---|---|
| | Contamination ratios: overall, per hospital and per product<br>Overall contamination ratio 57.7% (60/104 products) – stock solutions were not contaminated (*note: breakdown between numbers of stock versus diluted solutions not given*)<br>Contamination among hospitals ranged from 0 to 100% (*note: low numbers per hospital)*<br>Contamination among products ranged from 41.6% (10/24) for tar acid phenol/cresol to 3/3 (PCMX), ratio for CHG-QUAT (Savlon) was 68.0% (17/25)<br>Stock (undiluted) disinfectant solutions; no growth<br>Colony counts in contaminated products:<br>Mean contamination counts per hospital ranged between 3.1 $10^1$ and 1.6 $10^4$ CFU/ml<br>Contaminating flora (on 67 isolates), for all products combined:<br>*E. coli* 19 (28.4%); *P. aeruginosa* 16 (23.9%); S. *aureus* 15 (22.4%), *Proteus* spp. 11 (16.4%); *Bacillus subtilis* (*Bacillus spizizenii*) 6 (9.0%) – *note: no specification per product mentioned* | Risk factors<br>Assumed:<br>Contamination arose during dilution (as stock vials were not contaminated)<br>Over-dilution<br>Inappropriate diluents: tap water<br>Large containers<br>Topping-up<br>Too long in-use<br>Poor level of hygiene of operators |
| Zhang 2008<br>China (MIC)<br>Tertiary hospital | Objective and Design: routine infection control monitoring, anecdotal observation.<br>Product: disinfectants<br>Alcohol 75% in-use in the pediatric ward and the pharmacy<br>Contaminating flora: *Bacillus* spp. (*Bacillus amyloliquefaciens).*<br>Cultures of raw products (alcohol 95% and distilled water used for dilution) were sterile. Cultures of the inner surfaces of containers and funnels used yielded similar bacteria. | Methods<br>Sample selection not described. Routine sampling, nutrient agar.,<br>VITEK-32 identification; AST: disk diffusion, guidelines not mentioned<br>Resistant to penicillin, ampicillin, minocycline, azithromycin, erythromycin, tetracycline and trimethoprim-sulfamethoxazole<br>Risk factors:<br>Assumed: most probably contamination during preparation. Observed other risk practices: containers not covered by lids, topping-up, use beyond expiry date |

| | | |
|---|---|---|
| El-Mahmood 2009<br>Nigeria (MIC)<br>Tertiary hospital | Objective: to assess the contamination of products and the resistance of the contaminating flora to antibiotic and antiseptics<br>Design: Cross-sectional study, commonly used disinfectants (n = 100 from 5 brands) in the operation theatres, patients' wards, and accident/emergency units of the hospital; no specification of the breakdown of diluted and undiluted products. In-use and stock products.<br>Products and Contamination ratios per product: disinfectants<br>Stock solutions showed no bacterial contamination<br>In-use samples had an overall 20% contamination ratio.<br>Purit (*CHG-QUAT*): 30% (6/20): *S. aureus*, *P. mirabilis* and E. *coli* at 6.3 $10^7$ CFU/ml<br>Dettol (*PCMX, 4.8%*): 25% (5/20): *P. aeruginosa* and *E. coli* at 5.4 $10^8$ CFU/ml<br>Parazone (*chlorine*) : 20% (4/20) : *S. aureus*, *P. mirabilis, P. aeruginosa* at 7.1 $10^7$ CFU/ml<br>Z-germicide (tar acid phenol, *phenol*) : 20% (3/15) : *S. aureus* and *E. coli*, 4.9 $10^6$ CFU/ml<br>Septol (chloro-hydro-diphenyl methane, *phenol*) : 20% (2/10) : *S. aureus, P. aeruginosa*, 7.5 $10^5$ CFU/ml | Methods<br>Sample selection not described<br>Sampling: (*modified Kelsey-and-Maurer method*): 1 ml in 9 ml peptone broth, triplicate inoculation (1 ml and log10 dilutions) on nutrient agar (37°C, 48h), colony counting<br>ID: conventional phenotypic methods<br>AST: disk diffusion, guidelines not mentioned - no denominator (total isolates). (Note: *selection antibiotics not tailored to the organism, inconsistent results (e.g. (P. aeruginosa susceptible to ampicillin and trimethoprim-sulfamethoxazole, S. aureus susceptible to ampicillin)*<br>Risk factors<br>Observed<br>Preparation in the wards by unqualified ward attendants with little or no supervision by the staff nurses<br>Over-dilution (CHG), tap water, large containers, prolonged use<br>Assumed<br>Selection of multi-resistant strains by residual products |
| Muchina 2009<br>Kenya (LIC)<br>Primary, secondary and tertiary hospitals (n = 5) | Objective: Routine microbiological quality control of the disinfectants, part of a larger study assessing infection control and prevention in public maternity units in Kenya<br>Design: A total of 20 samples from maternity units in 5 hospitals were sampled and processed.<br>Products: disinfectants (*note: product concentrations were not mentioned*<br>JIK (sodium hypochlorite, *chlorine*)<br>Lysol* Biotex* (*detergent*)<br>Presepts (sodium dichloroisocyanurate, *chlorine*) Cidex (*glutaraldehyde*) | Methods<br>Sample selection not described<br>Sampling 1 ml in 9 ml peptone broth, no neutralizer, inoculation on nutrient agar, incubation at 37°C for 3 days and room temperature for 7 days. Counts > 5 colonies = contamination (*modified Kelsey-and-Maurer*)<br>ID: conventional phenotypic method, no AST |

| | | |
|---|---|---|
| | Contamination ratios per hospital and product:<br>Overall contamination ratio was 60% (12/20), varying from 0/3 samples to 6/6 samples across the 5 hospitals.<br>Hypochloric acid (no concentration specified): 7/15 samples contaminated<br>Biotex* (2 samples), Lysol* (1 sample), Cidex (1 sample): no contamination<br>Precepts (1 sample) contaminated<br>Contamination ratio according to time of use:<br>Contamination ratios increased with increasing in-use time (duration)<br>Less than 6 h post-dilution: 2/6 failed the in-use test<br>After 6 h post-dilution: all failed the in-use test<br>Contaminating flora: Hemolytic staphylococci, *Staphylococcus*, Enteric bacilli, *Proteus* spp., *Pseudomonas* spp., airborne contaminants | Risk factors:<br>Observed<br>Preparation by non-trained staff<br>Not clearing containers before refill<br>No lid to cover the containers<br>Over-dilution<br>Prolonged use (longer than 6 hours recommended by manufacturer) |
| Deress 2014<br>Ethiopia (LIC)<br>Tertiary hospital | Objective: to assess the contamination of products<br>Design: A total of 86 products from different units of the hospital were sampled and processed<br>Products and Contamination ratios and flora per product: antiseptics/disinfectants<br>Overall contamination ratio 3.5% (3/86)<br>Alcohol (Ethanol 70%): 4.0% (1/25): *K. pneumoniae*<br>Tincture Iodine 2% : 0.0% (0/14)<br>Savlon 2% (CHG-Cetrimide, *CHG-QUAT*) : 0.0% (0/14)<br>Hydrogen peroxide 3%: 0.0% (0/12)<br>Bleach 0.05% (*chlorine*): 9.3% (2/21): *K. pneumoniae* (n = 2) and *K. ozaenae* (n = 1) | Methods<br>Sample of all types of antiseptics/disinfectant<br>*Modified Kelsey-and-Maurer method*: 1 ml in 9 ml peptone broth, incubation at 37°C for 24h, subculture on MacConkey agar at 37°C for 24 – 48 h<br>ID by conventional phenotypical methods<br>AST: disk diffusion, NCCLS – the version not mentioned<br>*K. pneumoniae* resistant to ampicillin, amoxicillin-clavulanic acid, chloramphenicol and gentamicin (*note: cephalosporins were not tested)*<br>Risk factors<br>Observed<br>Use of tap water for dilution<br>Correct storage and use within expiry date<br>Use of recycled containers (original and non-original) |

| | | |
|---|---|---|
| Singh 2014<br>India (MIC)<br>Tertiary hospital | Objective: to assess the contamination of products<br>Design: cross-sectional survey of disinfectants and antiseptics, 100 samples were collected in various wards including outpatient department, indoor patient department, major and minor operating theatres and blood bank.<br>Products and Contamination ratios and flora per product: disinfectants<br>Intalon (Savlon) (CHG-Cetrimide, *CHG-QUAT)* : 1/67<br>Glutihyde (glutaraldehyde 2%): 1/11 (note: concentration was too low)<br>Betadine (*povidone iodine*): no contamination<br>Mega ciprit (*isopropyl alcohol 70%*): 9/9 contaminated<br>Sodium Hypochlorite 1/1 (*chlorine*, note: concentration was too low)<br>Contaminating flora: *P. aeruginosa* was isolated from all grown samples | Methods<br>Samples selected based on acceptability and frequency of use<br>Kelsey-and-Maurer method, neutralizer (3% Tween 80), duplicate subculture (10 x 0.02 ml drops) in duplicate on nutrient agar (1 x at 37°C for 3 days, 1 x at room temperature for 7 days. Growth in more than 5 drops was considered as contamination<br>ID: Conventional phenotypical methods<br>No AST done<br>Risk factors:<br>Observed<br>Only 24/100 samples had correct dilutions<br>Assumed<br>Organic materials, Prolonged use, Dilution with tap water |
| Akabueze 2015<br>Nigeria (MIC)<br>Secondary hospitals (n = 20) | Objective: to assess the contamination of products<br>Design: 92 products from 20 hospitals: floor-mop buckets, cleansing, discard jars and disinfectant solutions in which instruments, crockery and bed pans were rinsed, and closed containers of disinfectants<br>Contamination per product at given in-use time (numbers): disinfectants<br>Days 1 – 7 / Days 8 – 14 / Days ≥ 15<br>Purit (CHG-Cetrimide, *CHG-QUAT* 1/5 5/8 6/7<br>Spirit (70% ethanol/isopropyl) 1/6 2/4 6/10<br>Izal (Tar Phenol 7%) 1/4 4/8 6/8<br>Dettol (*PCMX*) 4.8% 0/2 1/4 4/6<br>Bleach (Sodium Hypochlorite, *chlorine*) 6% 2/6 4/9 4/5<br>Contamination ratios and flora per product: over-time testing of contamination<br>Overall contamination ratio was 51.1% at Day 7<br>Contaminating flora from the 47samples failing the in-use test: *P. aeruginosa* 36.2%, coagulase negative staphylococci 29.8%, *Staphylococcus aureus* (19.1%), *Proteus* spp. 14.1%<br>Culture from diluted disinfectants in closed containers did not grow | Methods<br>Random sampling<br>Kelsey-and-Maurer method: 1ml in 9ml Ringers' solution, neutralizer (0.5% sodium thiosulfate). Inoculation of 10 drops of 0.020 ml on 2 agar plates, incubation at 37°C and at room temperature for 72h respectively). Positive test (= contamination) at 1000 CFU/ml.<br>ID: conventional phenotypical tests<br>No AST done<br>Risk factors<br>Observed<br>Prolonged use (no recommendation about shelf-life of products) |

| | | |
|---|---|---|
| Firesbhat 2021<br>Ethiopia (LIC)<br>Tertiary hospital | Objective: to assess high-touch surfaces, left-over vials and antiseptics for bacterial contamination<br>Design: 40 in-use left-over alcohol-based (ethanol and alcohol-based hand rub) samples collected in addition to high touch surfaces and left-over medication in surgical, post-natal, orthopedic, trauma wards, and neonatal intensive care unit<br>Products: antiseptics<br>Ethanol 80%<br>Alcohol based hand rub (ethanol 80%, according to World Health Organization's formula)<br>Contamination ratios and flora per product:<br>Contaminating flora: 4/40, alcohol-based products were contaminated, comprising *A. baumannii* (2/4), coagulase-negative Staphylococcus (1/4) and *Bacillus* spp. (1/4). | Methods<br>Sample size calculated using the expected prevalence of 50%, precision level 5% and confidence interval 95%<br>Pre-incubation in tryptone soy broth at 37°C for 24 h, Centrifugation (3000 rpm for 3 min.), and subculture the sediment on blood, MacConkey, Chocolate agar and MSA, incubation at 37°C for 24 – 48 h<br>ID by conventional phenotypic methods<br>AST: disk diffusion, CLSI 2021<br>*A. baumannii* isolates (n = 7 in total) were susceptible to ciprofloxacin (88.9%), amikacin (88.9%), trimethoprim-sulfamethoxazole and imipenem (77.8%)<br>Risk factors: not mentioned |

*The following products were not included for analysis:
Acriflavine: acridine dye with antimicrobial properties , rarely used in wound care [34]
Mercurochrome (brand name): different products with different ingredients marketed; the original branded product contained mercury and is no longer used as an antiseptic in US and Europe
Methimasol: no information retrieved ("methimazole" is a medicine used for treatment of hyperthyroidism)
Lysol (brand name): generic component not mentioned in the article, there are different commercial products named "Lysol" with different ingredients marketed [35]
Biotex (brand name): (household) detergent, generic component(s) not mentioned
Harpic (brand name): cleaning product, generic component(s) not mentioned, different products marketed