Microbial status and product labelling of 58 original tattoo inks

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Abstract

Background European Council resolutions on tattoo ink introduce sterility and preservation of inks to protect customers. Inks used in Denmark are typically purchased over the internet from international suppliers and manufacturers from the US and the UK. In Denmark tattoo inks are regulated and labelled according to REACH as if they were plain chemicals.

Objective The objective of this study was to check the microbial product safety of unopened and opened tattoo ink stock bottles. Packaging, labelling, preservation, sterility and contamination with micro-organisms were studied. **Methods** Physical inspection and culture of bacteria and fungi.

Results Six of 58 unopened stock bottles (10%) were contaminated with bacteria and one of six samples (17%) of previously used stock bottles was contaminated. The bacterial species represented bacteria considered pathogenic in humans as well as non-pathogenic environmental bacteria. Yeast or moulds were detected in none of the samples. A total of 31% of the manufacturers informed only about the brand name. No information about content, sterility, risks or expiry date was indicated on the label. A total of 42% claimed sterility of their inks. A total of 54% labelled a maximum period of durability of typically 2–3 years. The physical sealing was leaking in 28% of the products.

Conclusions The European Council resolutions regarding safety of tattoo inks are not effective. Stock bottles of tattoo ink may contain bacteria pathogenic to humans and environmental bacteria, and packaging, labelling and preservation of inks are of inadequate quality. Claim of sterility can be erroneous. Received: 3 May 2011: Accepted: 7 November 2011

Conflict of interest

None.

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Introduction

Today tattoos are mainstream, omnipresent and socially accepted across class barriers. In Denmark recent surveys showed that 13% of adults are tattooed, and 41% of these have more than one tattoo.^{1–3} Tattooing is subject to little or no formal control by national or European Community regulations. An American study found that up to 24% of persons between 18 and 50 years of age were tattooed.⁴ The tattoo business is international.

The Council of Europe, an advisory organization without legal power, over the last decade has made two recommendations with regard to the safety of tattooing. The two proposals differ in the aspect of preservation and disposable containers. In Resolution ResAP(2003)2 it is stated that tattoo products should be manufactured as sterile single-use containers without preservatives.⁵ Contrarily, the Resolution ResAP(2008)1 legalizes the use of preservatives and multi-use containers without providing sufficient details on how to preserve.⁶ The field remains uncertain. The resolutions of the Council of Europe are drafted for legal implementation, however, elements of the resolutions have hitherto been implemented in Germany, Switzerland and The Netherlands only.

Cases of infective complications following tattooing are reported in the literature.^{7–10} The actual epidemiology of infections is not known. It is difficult to determine the true incidence of tattoo-related infections as few patients may consult their physicians regarding minor cases, opting instead to return to the tattoo parlour.¹¹ Kazandjieva *et al.* estimated the overall prevalence of

tattoo complications to be 2.1% (five of 234 cases) including infections, allergic and/or granulomatous complications in tattoos.¹² A recent German survey based on an internet sample with 3411 spontaneous responders investigated the possible health risks associated with tattoos.¹³ About 0.4% reported pus-filled tattoo lesions and 1.1% reported fever directly after tattooing, health problems that might be due to bacterial infection. The survey may not be accurate regarding infections and under-report.

A wide range of micro-organisms including bacteria, virus and fungi have been associated with installation of tattoo inks in the skin e.g. Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa and Mycobacteria.⁷⁻¹⁰ Inoculation of syphilis was a known complication of tattooing in old days mostly related to the use of the tattooist's saliva during the procedure.¹⁴ Severe cutaneous infections apparently occur in amateur tattooing or traditional tattooing as exemplified in New Zealand.9,15 Cases of life-threatening cellulitis or necrotizing fasciitis from New Zealand were reported following traditional tattooing.^{16,17} Improper sanitary conditions and primitive inks in combination with late presentation to medical services have been suggested as causative. In 2004, an epidemic of cutaneous infections with methicillin-resistant S. aureus was reported in the USA following tattooing performed by unlicensed tattooists.¹⁸ Few cases of systemic infections following professional tattooing have been published such as cases of endocarditis associated with tattooing in patients with congenital heart failure.19,20

During the past few years several outbreaks of atypical mycobacterial infections, especially *Mycobacterium chelonae* in tattoo parlours have been reported. Case reports of *M. chelonae* in healthy adults presented with skin elements confined to the grey parts of their tattoos.^{21–23} The grey wash was prepared by dilution of black pigment with tap water. Use of tap water mixed with the ink by the tattooist may be the cause of such contamination.²¹ Tap water is considered the major reservoir for most human nontuberculous mycobacteria pathogens.²² Similarly, few cases of *Mycobacterium haemohpilum* skin infection in tattooed healthy adults have recently been reported.^{24,25} Although the environmental reservoir for *M. haemophilum* is not known, water has been a suspected reservoir.²⁴

However, even with hygiene and sanitation measures taken infections may occur. Infection from tattooing may be related to transmission from the tattooist, contaminated needles, equipment, utensils or to contamination of the inks. In 2004, in Belgium, 'Starbrite Colours' tattoo ink was withdrawn from the market due to microbial contamination with *P. aeruginosa.*²⁶ In Switzerland, in 2009, a follow-up report after the introduction of the European Council recommendations concluded that 3% of inks from tattoo parlours were contaminated.²⁷ Kluger *et al.* conducted a bacteriological analysis of 16 tattoo inks which had previously been opened and were in current use.²⁸ None of the samples grew positive for pyogenic bacteria, mycobacteria or fungi in their setup.

Indeed, bacteria have been cultured from previously unopened stock bottles.^{29–31} In 2001 the Inspectorate for Health Protection, The Netherlands, carried out a survey on the microbiological condition of tattoo- and permanent make-up inks.³⁰ Eleven samples (18%) were contaminated with microbes. Eight samples were from opened bottles under usage and three from sealed bottles. Ten per cent of the sealed bottles were contaminated. *Pseudomonas* species inclusive *P. aeruginosa* were identified. A similar study in the Principality of Liechtenstein analysed 145 samples (34%) were contaminated. Seventeen (43.6%) were colours form new and sealed vials and 32 (30.3%) from opened vials.³¹ Charnock also found bacteria in unopened and opened stock bottles.²⁹

In Denmark, tattoo inks are regulated and labelled in accordance with the REACH regulation of the European Union addressing chemicals in contact with body surfaces. European Council regulations of tattoo ink are not implemented in Denmark. Tattoo inks are typically purchased over the internet from international suppliers and manufacturers from the US and the UK, thus, production and sales do not automatically fall under European or local law. The marketplace is dynamic with Asian suppliers entering. This study was undertaken to analyse the microbial product safety of tattoo inks in terms of packaging, labelling, preservation and sterility. The study was based on newly acquired stock bottles of ink.

Materials and methods

Guided by a thorough market analysis carried out by the Danish Ministry of the Environment we purchased 58 commonly used tattoo inks, i.e., red, blue, green, yellow, white and black colours, from 13 different manufacturers.³² The inks were purchased on the internet during February 2010 and the last analysis was performed in July 2010. The manufacturers were Dynamic Color Co. (Ft. Lauderdale, FL, USA), Intenze (South Rochelle Park, NJ, USA), Wefa Color (Lahnstein, Germany), Eternal Ink (Brighton, MI, USA), Gold (Moreton, UK), Huck Spaulding Enterprises, Inc., VooDoo (Voorheesville, NY, USA), Starbrite, (Ft Lauderdale), Micky Sharpz, Easyflow (Birmingham, UK), National Tattoo Supply, homogenized tattoo ink (Allentown, PA, USA), Yakusa (San Ferdinando di Puglia, Italy), Tattoo Inkorporated (manufacturer not declared), Talens Black Ink (Apeldom, Holland) and Pelikan Ink (Hannover, Germany). Talens Black Ink is used by tattooists against manufacturers' advice about intended use.

The physical packaging of the purchased inks was examined on receipt and registered with emphasis on the condition of the sealing. Composition, claims about sterility and preservation and expiry dates were read from product labels or from material safety data sheets (MSDS).

In addition, we were given six original stock bottles of tattoo ink from tattooists of clients referred to our clinic as patients with problem tattoos. Three patients had lichenoid reactions with inflammation in their tattoos, two had inflammatory reactions and one had a granulomatous reaction. Samples of the tattoo inks were taken under sterile conditions and cultured for bacteria and fungi as described below.

Bacteria

Samples were examined for total count of bacteria and bacterial species. The microbiological tests were performed by the Department for Microbiological Surveillance and Research, Statens Serum Institut (SSI), Copenhagen, Denmark. A volume of 100 µL tattoo ink was transferred to 5% blood agar plates (SSI Diagnostics, Hillerød, Denmark; incubated at 35 °C/CO₂), anaerobic plates (SSI Diagnostics; incubated under anaerobic conditions in an anaerobic chamber at 35 °C) and serum broth (SSI Diagnostics, Hillerød, Denmark; incubated at 35 °C). All samples were incubated for 3 days. Only bacteria that could be found in more than three colonies e.g. >30 colonies/mL, were typed. The remaining cultures were considered as contamination. Typing of bacteria was performed by microscopy of Gram coloured preparations, selective growth on relevant agar plates and mass spectroscopy (MaldiTof, Microflex, MaldiTheory Mass Spectrometry, Bruger Daltonik GmbH, Bremen, Germany).

Fungi

Samples of tattoo ink were inoculated on Sabouraud-glucose-agar plates (VWR – Bie & Berntsen A/S, Herlev, Denmark) and V8 agar plates (Danish Institute of Technology, Taastrup, Denmark,³³) and incubated at 20 °C for at least 12 weeks. The tattoo ink was considered contaminated with fungi if a fungus grew from all three inoculation sites on the agar plate and when growth of the same fungus was confirmed by re-inoculation. Fungal isolates were identified by classical phenotypic criteria.^{34,35} Isolation and diagnosis of fungi was performed at the Laboratory of Department of Dermatology, Copenhagen University Hospital, Bispebjerg, Denmark.

Table 1 Bacteria isolated in purchased inks

Results

The purchased inks were aqueous dispersions ready-to-use with water as the main vehicle except for one supplier, who sold tattoo inks in powder form with vodka as a recommended vehicle. None of the products contained a written insert with additional information.

Bacteria

In total, significant growth (\geq 3 colonies/plate) was found in 7 of the 64 (11%) tested tattoo inks. Six originated from unopened stock bottles and one from a previously opened and used stock bottle delivered by a patient with a problem tattoo, i.e. lichenoid reaction following a complicated inflammatory initial phase where infection might have been active. In other words 6 of 58 unopened stock bottles (10%) were contaminated with bacteria and one of six samples (17%) of previously used bottles was contaminated. The number of bacteria in positive samples was at least 100 colonies/mL. The collected bacteria included bacteria of clinical relevance and non-pathogenic environmental bacteria.

From unopened stock bottles the isolated bacteria of clinical importance were *Streptococcus sanguinis*, *Staphylococcus* sp., *Pseudomonas* sp., *Enterococcus faecium* and *Acinetobacter* sp. Likewise, *Streptococcus sanguinis* and *Acinetobacter* sp. were isolated from a used bottle in addition to *Streptococcus salivarius*. Table 1 shows the isolated bacteria of the purchased inks. It is not known how long the previously opened bottles were unsealed and to what extent the ink had been used for clients.

Fungi

In none of the samples yeast and moulds were detected. Species of *Penicillium* were isolated from tattoo inks Midnight blue and Tribal black (manufacturer 7, see Table 2) on the Sabouraud-glucose-agar plate; however, the growth was not confirmed by re-inoculation and thus considered a contamination.

Brand	Bacterial growth	Colonies per mL
Tattoo inks, samples delivered from suppliers		
LFG-green, Dynamic Color Co. (Ft. Lauderdale, FL, USA)	Bacillus sp. Staphylococcus sp.	100
True Blacks, Intenze (South Ochelle Park, NJ, USA)	Aeromonas sobriae Acidovorax	Ca. 650
Mediterranean Blue, Wefa Color (Lahnstein, Germany)	Streptococcus sanguinis	>500
	Pseudomonas sp.	
Lightning Yellow, Eternal Ink (Brighton, MI, USA)	Acinetobacter sp.	110
Midnight Black, VooDoo, Huck Spauldbing Enterprises, Inc. (Voorheesville, NY, USA)	Dietzia maris Blastomonas sp.	140
Black Ink, Talens (Apeldom, Holland)	Enterococcus faecium	Ca. 500
Tattoo inks, sample received from tattooist		
Indian Ink (supplier not identified)	Streptococcus salivarius	Ca. 500
	Streptococcus sanguinis	
	Acinetobacter sp.	
	Actinomyces sp.	

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Brand	Compositio	Composition and claim		Produc	Product expiry	Sealing	Sealing of bottle
	Vehicle	Claim of sterility	Claim of preservatives	Duration from production	Duration from opening	Method	Intact upon reception
(1) YD-2,BD-1, BLK	Acrylic resin,* Isopropanol	No	No	Not declared	Not declared	Membrane	Yes
(1) LFG,WD-1	Isopropanol*	No	No	Not declared	Not declared	Membrane	Yes
(1) RD-1	Acrylic resin, Isopropanol*	No	No	Not declared	Not declared	Membrane	No
(2) Lemon Yellow, Mario's Light Blue, Light Green	Water, glycerin, Isopropanol proprietary	Yes†	Not	∼4 years from reception§	Not declared	Membrane	Yes
(2) Bright Red, True Blacks	Water, glycerin, isopropanol, witch-hazel, proprietary	Yes†	No‡	∼4 years from reception§	Not declared	Membrane	Yes
(2) Snow White Opaque	Water, glycerin, isopropanol, witch-hazel, proprietary	Yes†	No‡	∼4 years from reception§	Not declared	Membrane	No
(3) Lemon Yellow, Draculas Dream	Water, PVP, shellac, propylenglycol, isoproponol	Yes†	Yes	2 years	12 months	Plug	No
(3) Mediterranean Blue, Snow White	Water, PVP, shellac, propylenglycol, isoproponol	Yes†	Yes	2 years	12 months	Plug	Yes
(3) Lime Green, Black Magic	Water, PVP, shellac, propylenglycol, isoproponol	Yes†	Yes	2 years	Not declared	Plug	Yes
 (4) Lightning Yellow, Light Red, Graffiti Green, White, Lining Black 	Distilled water, alcohol, witch-hazel	Yes**	No	3 years	3 months	Plug	No
(4) True Blue	Distilled water, alcohol, witch-hazel	No	No	~2 years from reception§	12 months	Plug	No
(5) Yellow Snow, Barely Legal Blue, Cheeky Cherry, Alien Green, Ghost White, Bad Ass Black	Purified water, witch-hazel, isopropanol	Yes†	Not†	2 years	Not declared	Plug	Yes
(6) Sun Yellow, Horizon Blue, Apple Green, Midnight Black	Water,'trade secret'‡‡	No	No	Not declared	Not declared	Membrane	Yes
(6) Blood Red	Water,'trade secret'#	No	No	Not declared	Not declared	Membrane	No
(6) Artic White	Water‡‡	No	No	Not declared	Not declared	Membrane	Yes
(7) Canary Yellow, Midnight Blue, Crimson Red, Jade, Brite White, Tribal Black	Distilled water, alcohol, glycerin	No	No	2 years	Not declared	Plug	Yes
(8) Light Yellow, Dark Blue, Deepest Red, Forest Green, Finest WhiteTribal Black	Water,glycerin, isopropanol, surfactant	No	Yes§§	\sim 2 years from reception§	Not declared	Membrane	Yes
(9) HLC10, HLC8, HLC2, HLC12	Not known	No	No	Not declared	Not declared	Membrane	No
(9) HLC19, HLC1	Not known	No	No	Not declared	Not declared	Membrane	Yes

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Brand	Composition and claim	n and claim		Produc	Product expirv	Sealing of bottle	f bottle
	Vehicle	Claim of	Claim of	Duration from	Duration from	Method	Intact upon
		sterility	preservatives	production	opening		reception
(10) Evil Red	Water, glycerin, witch-hazel, isopropanol	Yes**	No††	2 years	12 months	Membrane	Yes
(11) Red	Not known	No	No	Not declared	Not declared	Plastic bag	Yes
(12) Indian-Talens Liner Ink	Not known	No	No	Not declared	Not declared	No sealing	No
(13) Pelikan-drawing Ink	Not known	No	No	Not declared	Not declared	Plug	Yes
*5% acrylic resin and 10% Isopropanol, from MSDSs.	SDSs.						
†Method of sterilization not known.							
‡The label on the bottle reads 'proprietary' that may cover preservatives, not known.	it may cover preservatives, not kno	wn.					
SDate of production not declared.							
Ilodoproponyl-Butylcarbamate, Polyaminopropyl-biguanide.	yl-biguanide.						
**Gamma irradiation.							
ttlt is specifically notified that the items are without preservatives.	ithout preservatives.						
‡ ‡According to the MSDSs the items contain low levels	ow levels of resin or surfactant.						
§§Content not specified.							
A sealed plastic bag.							
Number in brackets indicate manufacturer:							
(1) Dynamic Color Co. (Ft. Lauderdale, FL, USA).							
(2) Intenze (South Rochelle Park, NJ, USA).							
(3) Wefa Color (Lahnstein, Germany).							
(4) Eternal Ink (Brighton, MI, USA).							
(5) Gold (Moreton, UK).							
(6) Huck Spaulding Enterprises, Inc., VooDoo (Voorheesville, NY, USA).	/oorheesville, NY, USA).						
(7) Starbrite, (Ft Lauderdale, FL, USA).							
(8) Micky Sharpz, Easyflow (Birmingham, UK).							
(9) National Tattoo Supply, homogenized tattoo ink (Allentown, PA, USA)) ink (Allentown, PA, USA).						
(10) Yakusa (San Ferdinando di Puglia, Italy).							
(11) Tattoo Inkorporated (manufacturer not declared).	clared).						
(12) Talens Black Ink (Apeldom, Holland).							
(13) Pelikan Ink (Hannover, Germany).							

Table 2 Continued

Packaging and labelling

A review on physical packaging, expiry dates and written product information about sterility and preservation of the panel of tested tattoo inks from previously unopened stock bottles is provided in Table 2.

Tattoo inks originated from 13 manufacturers. Four of 13 manufacturers (31%) only informed about the brand name. No information about content, sterility, risks or expiry date was indicated on the label (manufacturers: 1, 11, 12 and 13, Table 2). Requested MSDSs from all 13 manufacturers were received in only three instances (from manufacturers: 1, 6 and 8).

Five of 13 manufacturers (42%) claimed sterility of their inks. Two of 13 manufacturers (15%) labelled use of preservatives. Only one specified the chemical name of the preservative. Eight of 13 manufacturers (62%) declared their products to contain alcohol, i.e. isopropanol when specified. The quantity was indicated in one product series to be 10% with no information whether it was v/w or v/v. In the remaining cases it was not known if the products contained alcohol and what the recipe was.

Water, which is supposed to be the main part of the vehicle, was mentioned as distilled, purified (method not specified) or simply water. The presence of other additives was not specified and cannot be ruled out.

The content of pigments in the 58 samples was specified and expressed by the colour index number (CI) except for four cases (manufacturers: 9, 11, 12, 13). In only one sample the quantity of pigment was given, expressed simply as 10% (manufacturer 1). Thus, the concentration of pigment and dry materials was generally not known, and the recipes remained obscure.

Seven of 13 manufacturers (54%) labelled some maximum period of durability but only three implied reduced durability upon opening of the stock bottles, ranging from 3 to 12 months. The declared durability from date of production was 2–3 years. Two manufacturers did not declare the date of production but anyhow labelled a maximum period of durability of up to approximately 4 years measured from customers' reception of the product.

The physical sealing was not intact upon reception in 16 of 58 products (28%), see Fig. 1 The sealing, a plug, of all six bottles from one manufacturer was defective (manufacturer 4). Sealing methods involved plugs, membranes and in one case a sealed plastic bag containing tattoo ink in powder form. One product was not sealed at all and had clearly been filled from another container probably from a larger original container, Talens Black Ink (manufacturer 12). It carried the logo of the supplier as the only labelled information.

We contacted all 13 manufacturers and/or the suppliers of all purchased tattoo inks in request of sterilization methods independent of their labelling of sterility. We received specified sterilization information on two product series (manufacturers: 4 and 10). Both products were declared to be sent off for sterilization by gamma irradiation, however, the suppliers did not respond to a request on where sterilization was undertaken and by whom. Thus, information was not verified. One manufacturer (8) replied



Figure 1 Example of inadequate sealing of a tattoo ink stock bottle at receipt by customer. Broken membrane with leakage of red tattoo ink.

there was no sterilizing process as the inks contained preservatives, not specified, and isopropanol, quantity not known. Another manufacturer (1) wrote 'the inks are considered sterile, but not sterilized and without added preservatives'. They added alcohol to make it sterile, with 10% isopropanol in final product.

The vast majority of manufacturers did not indicate or express that production was up to any recognized good manufacturing standard or any recognized accreditation system or quality control. Manufacturers in general took no legal responsibility for their products and some manufacturers might as a very visible part of the labelling deliberately transfer the responsibility to the user of the product, i.e. the tattooist, see Fig. 2 Six disclaimed their responsibility (manufacturer 4, 5, 6, 7, 10, 11). Typically they wrote: 'The company specifically disclaims any responsibility for any allergic reaction of any individual to whom this dye or pigment is applied, whether implied by warranty of merchantability or fitness for purpose or otherwise when there is no history of a tolerance to this dye or pigment, spot testing or consulting a competent dermatologist prior to application is urged.'

Six manufacturers (46%) (manufacturers: 3, 4, 5, 6, 7 and 10) informed about potential adverse events, all in the form of a brief statement about 'allergy'. Four advised spot testing before application (manufacturers: 2, 5, 6 and 10). Six manufacturers (manufacturers: 1, 2, 5, 6, 9, 10) labelled that the products should not be applied in or near the eyes.

Four manufacturers (31%) informed about recommended storage conditions, i.e. 'keep inks in a cool and dry area' (manufacturers: 1, 4, 6 and 7).



Figure 2 An exceptional example of renouncement of any legal responsibility by the manufacturer with transfer of the full responsibility to the tattooist, if purchased. The ink is sold over the internet by a tattoo ink supplier operating from the UK.

Discussion

Eleven per cent of the analysed inks were contaminated with bacteria. In none of the samples yeast and moulds were detected. We did not study contamination with viruses and other micro-organisms, and we did not culture for mycobacteria. The labelling was widely inadequate or misleading and the packaging/sealing was defective in many cases. Requirements of the Council of Europe were not respected. Our findings are in accordance with the study of Baumgartner *et al.*³¹

Intradermal injection of contaminated tattoo products might result in serious infections. In the literature *Pseudomonas* species are the most common bacteria isolated from tattooing solutions.³⁶ *P. aeruginosa* is an important nosocomial and opportunistic pathogen. We found *Pseudomonas* sp. in one unopened stock bottle. Other examples of isolated strains in our study were *Staphylococcus* sp., *Streptococcus salivarius* and *Streptococcus sanguinis*. In the literature there are a few serious cases of pseudomonas infection and staphylococcus infection following tattooing, however, to our knowledge, there are no published reports on complicating infections related to other strains detected in our study.^{16,19,20,37}

The Talens Black Ink product supposed to be filled from a larger original container into small containers turned out to be heavily contaminated with *Enterococcus faecium*. It is most likely an example of contamination due to improper handling by the supplier. Enterococci are resistant to conventional antibiotics and are recognized as nosocomial pathogens that can be challenging to elucidate.^{38,39}

It is a remarkable finding and of much concern that only 42% of the manufacturers claimed their products to be sterile. This is in conflict with the resolutions of the Council of Europe reading that inks shall be sterile and guarantee of sterility shall be declared

on the packaging.^{5,6} Surprisingly, 3 of the 24 inks claiming sterility were, nevertheless, contaminated with bacteria. Thus, the sterility claim was misleading. Sterile production up to modern standard is a costly business that would be expected to increase the price of inks markedly. However, we noted no price difference among products declared sterilized and products not declared so. In addition, one of the two product lines declaring sterilization by gamma irradiation had sealing defects in all samples of the line upon reception and one of those samples was tested positive for *Acinetobacter* sp. Containers might be overfilled, and pressure changes during transport by plane might result in leakage.

According to the Council of Europe resolution of 2008 preservatives should only be used to ensure the preservation after opening and not as a correction of insufficient sterile production.⁶ Two manufacturers claimed use of preservatives. One product was positive for *Streptococcus sanguinis* and *Pseudomonas* sp. A total of 62% stated their products to contain alcohol. A content of 10% was declared in a single product series. However, used as disinfectant isopropanol is effective at 70% in water and little effective at 10%.⁴⁰ Consistently, we found microbiological contamination in 4 of 58 (7%) inks declared to contain alcohol.

Charnock tested the efficacy of the antimicrobial preservation of tattoo inks.³⁶ He conducted challenge tests to assess the survival of bacteria added to tattoo inks. He concluded that tattoo solutions in some instances can represent favourable environments for bacterial growth. He documented that *P. aeruginosa* can grow in some products.

Only 54% of manufacturers informed about durability. Some declared reduced durability upon opening ranging from 3 to 12 months. In one contaminated sample there was no labelled claim of sterility or use of preservatives but nevertheless a durability claim after opening of 1 year (manufacturer 4). In the field of pharmaceuticals a typical guideline for simple injectives produced as simple solutions in repeat use containers is maximum 28 days for injectives containing preservatives and <14 h if not preserved.²⁹

It is firmly expressed by the European Council that tattoo inks for the safety of customers shall be supplied sterile, a guidance not yet implemented as our study implicates.

There is a strong need to develop international control systems and regulations both addressing the manufacturing of inks, their distribution and tattooing practices. Present practice exposes the population to risks that are not taken with any other type of injective for human use. Tattoo inks are potential vectors for spread of new infections in the community.

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References

- Berlingske Tidende with reference to Gallup analyseinstitut. Tattoverings-boom blandt unge danskere. 2010. [WWW document] URL http:// www.berlingske.dk/danmark/tatoverings-boom-blandt-unge-danskere#! (last accessed 1 December 2010).
- 2 MetroExpress with reference to YouGov Zaperas danmarkspanel. Er du også skræmt over tatoveringer? 2010. MetroExpress. [WWW document] URL http://www.metroxpress.dk/dk/article/2009/09/15/20/5026-83/ index.xml. (last accessed 10 January 2011).
- 3 Serup J. Department of Dermatology, Copenhagen University Hospital, Bispebjerg, Denmark, 2010. Unpublished data.
- 4 Laumann AE, Derick AJ. Tattoos and body piercings in the United States: a national data set. J Am Acad Dermatol 2006; **55**: 413–421.
- 5 Council of Europe Com. Resolution ResAP(2003)2 on tattoos and permanent make-up. 2003. [WWW document] URL https://wcd.coe.int/ wcd/ViewDoc.jsp?id=45869 (last accessed 5 October 2010).
- 6 Counsil of Europe. Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up. 2008. [WWW document] URL http://www.camtattoo.cn/msds/eis_tattoo_risk_ 052003_en.pdf (last accessed 5 January 2010).
- 7 Papameletiou D, Zenié A, Schwela D *et al.* Risks and health effects from tattoos, body piercing and related practices. 2003. European Commision. [WWW document] URL http://www.camtattoo.cn/msds/eis_tattoo_risk_ 052003_en.pdf (last accessed 5 January 2010).
- 8 Kluger N. Cutaneous complications related to permanent decorative tattooing. *Expert Rev Clin Immunol* 2010; **6**: 363–371.
- 9 Nishioka S, Gyorkos T. Tattoos as risk factors for transfusion-transmitted diseases. *Int J Infect Dis* 2001; 5: 27–34.
- 10 Long GE, Rickman LS. Infectious complications of tattoos. *Clin Infect Dis* 1994; 18: 610–619.
- 11 Mataix J, Silvestre JF. Cutaneous adverse reactions to tattoos and piercings. Actas Dermosifiliogr 2008; 100: 643–656.
- 12 Kazandjieva J, Tsankov N. Tattoos: dermatological complications. *Clin Dermatol* 2007; 25: 375–382.
- 13 Klugl I, Hiller KA, Landthaler M *et al.* Incidence of health problemsassociated with tattooed skin: a nation-wide survey in german-speaking countries. *Dermatology* 2010; 221: 43–50.
- 14 Davis RG. Hazards of tattooing: report of two cases of dermatitis caused by sensitisation to mercury (cinnabar). US Armed Forces Med J 1960; 11: 261–280.
- 15 Nishioka S, Gyorkos T, Joseph L *et al.* Tattooing and risk for transfusion-transmitted diseases: the role of the type, number and design of the tattoos, and the conditions in which they were performed. *Int J Infect Dis* 2001; **5**: 27–34.
- 16 Porter CJW, Simcock JW, MacKinnon CA. Necrotising fasciitis and cellulitis after traditional Samoan tattooing: case reports. J Infect 2003; 50: 149–152.
- 17 McLean M, D'Souza A. Life-threatening cellulitis after tarditional Samoan tattooing. Aust N Z J Public Health 2011; 35: 27–29.
- 18 Centers for Disease Control and Prevention (CDC). Methicillin-resistant Staphyloccus aureus skin infections among tattoo recipients- Ohio, Kentucky, and Vermont, 2004–2005. MMWR Morb Mortal Wkly Rep 2006; 55: 677–679.
- 19 Tse D, Khan S, Clarke S. Bacterial endocarditis complicating body art. Int J Cardiol 2008; 133: e28–e29.
- 20 Satchithananda DK, Walsh J, Schofield PM. Bacterial endocarditis following repeated tattooing. *Heart* 2001; **85**: 11–12.
- 21 Kluger N, Muller C, Gral N. Atypical mycobacteria infection following tattooing: review of an outbreak in 8 patients in a French tattoo parlor. *Arch Dermatol* 2008; **144**: 941–942.

- 22 Drage LA, Ecker PM, Orenstein R et al. An outbreak of Mycobacterium chelonae infections in tattoos. J Am Acad Dermatol 2010; 62: 501–506.
- 23 Binic I, Jankovic A, Ljubenovic M et al. Mycobacterium chelonae infection due to black tattoo ink dilution. Am J Clin Dermatol 2011; 12: 404–406.
- 24 Kay MK, Perti TR, Duchin JS. Tattoo-associated Mycobacterium haemophilum skin infection in immunocompetent adult, 2009. Emerg Infect Dis 2011; 17: 1734–1736.
- 25 Hamsch C, Hartschuh W, Enk A *et al.* A chinese tattoo paint as a vector of atypical Mycobacteria-outbreak in 7 patients in Germany. *Acta Derm Venereol* 2011; **91**: 63–64.
- 26 Harp B. FDA: tattoos and permanent makeup. 2009. [WWW document] URL http://learningcenter.nsta.org/products/symposia_seminars/fall09/fda/files/WS4-12-17-09.ppt. (last accessed 2 November 2010).
- 27 Swiss Confederation. Conformity of tattooing- and permanent make-up-colours not satisfied. 2006. [WWW document] URL http:// ctl-tattoo.net/FOPH-Report_tattoo-colours_control-campaign.pdf (last accessed on 25 October 2010).
- 28 Kluger N, Terru D, Godreuil S. Bacteriological and fungal survey of commercial tattoo inks used in daily practice in a tattoo parlor. *J Eur Acad Dermatol Venereol* 2011; 25: 1230–1231.
- 29 Charnock C. Tattooing dyes and pigments contaminated with bacteria. *Tidsskr Nor Laegeforen* 2004; **124**: 933–935.
- 30 Reus H, van Buuren R. Kleurstoffen voor tatoeage en permanente make-up. (Inspectorate for Helath Protection North, Ministry of Health. Legislation, report no ND COS 012). 2001. [WWW document] URL http://www.tattoots.nl/images/pdf/Kleurstoffen.pdf (last accessed 25 October 2010).
- 31 Baumgarter A, Gautsch S. Hygienic-microbiological quality of tattooand permanent make-up colours. URL: http://www.springerlink.com/ content/q31162u817388128/.
- 32 Miljøstyrelsen. Undersøgelser af farvestoffer i tatoveringsfarver. Kortlægning 2002. 2002. [WWW document] URL http://www.mst.dk/ Virksomhed_og_myndighed/Kemikalier/Forskning_og_kortlaegning/ Kortlaegning+af+forbrugerprodukter/Rapporter4/02051100.htm (last accessed 5 February 2010).
- 33 Gravesen S. Identification and quantitation of indoor airborne microfungi during 12 months from 44 Danish homes. *Acta Allergol* 1972; 27: 337–354.
- 34 De Hoog GS, Guarro J, Gené J *et al. Atlas of Clinical Fungi*, 2nd edn. Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili, Reus, Spain, 2000.
- 35 Samson RA, Hoekstra ES, Frisvad JC. Introduction to Food- and Airborne Fungi, 7th edn. Ponsen and Looyen, Wageningen, The Netherlands, 2004.
- 36 Charnock C. Biocidal activity of a bioactive glass-protected, preservative-free tattooing solution. Am J Infect Control 2006; 34: 290–295.
- 37 Korman TM, Grayson ML, Turnidge JD. Polymicrobial septicaimia with *Pseudomonas aeruginosa* and *Streptococcus pyogenes* following traditional tattooing. *J Infect* 1997; **35**: 203.
- 38 Murray P, Rosenthal K, Kobayashi G et al. Medical Microbiology, 3rd edn. Mosby, St. Louis, Missouri, USA, 1998.
- 39 Fraser S, Lim J, Donskey C *et al.* Enterococcal infections. 2010. [WWW document] URL http://emedicine.medscape.com/article/216993-overview. (last accessed 3 December 2010).
- 40 Turpin K. Ethanol vs. Isopropyl Alcohol to disinfect. 2010. [WWW document] URL http://www.ehow.com/about_6540795_ethanol-vs_-isopropyl-alcohol-disinfect.html (last accessed 3 December 2010).