

Comprehensive Assessment of Microbiological and Bioaerosol Contaminants in Dammam Slaughterhouse, Saudi Arabia

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To assess indoor air quality in Dammam slaughterhouse, samples were taken from two different laboratories. Bioaerosols as well as some slaughterhouse utensils examination indicated that most common bacteria; *Salmonella* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., and *Bacillus* sp., while fungi include; *Aspergillus* sp., *Microsporum* sp., *Rhodotorula mucilaginosa*, *Candida* sp., and *Cryptococcus* sp., as tested on MacConKey, MSA, Blood agar and Sabouraud destrose agar media, for bacteria and fungi, respectively. Bacterial contaminants were exposed to antibiotic profiling and revealed that; *Streptococcus* sp. strain-AS10/7, *Staphylococcus* sp. strain-A/1 and *Staphylococcus* sp. strain-KB/1 showed highest resistance to streptomycin. While, *Streptococcus* sp. strain-AS5/1, showed highest resistance to ampicillin and erythromycin. Strains from air of Laboratory B (except; *Bacillus* sp. strain-AS2/11) showed the highest sensitivity towards all tested antibiotics. Heavy metal tolerance revealed that, all strains isolated from air of Laboratory B were moderately resistant to Ag⁺ and highly sensitive to lower concentration of Hg⁺ and Cr⁺⁶. Some bacteria showed B-haemolytic activity, reflecting serious risk to human health. For disinfection, 10% Clorox solution was less effective while, Dettol solution (10%) showed the highest effect, except with the most resistant *Staphylococcus* sp. strain-AS5/2, thus recommended for human health and safety.

Key words: Slaughterhouse, indoor air, bioaerosol, antibiotics profiling, heavy metals profiling.

There is growing interest among different governments concerning public awareness in different sectors related to human health for protection against diseases. Recently, environmental problems caused by slaughtering activities attract the attention of competent administrative authorities and environmental scientists as well. Indeed, assessment of slaughterhouses showed high hazard which directly affects the hygienic quality and microbiological specifications of the produced meats.

Air pollution, both indoor and outdoor, is often considered one of as the major cause of environmental health problems. Outdoor air pollution has been well publicized due to the major significance of pollutant sources¹. Recently, public concerns on indoor air quality (IAQ) have drawn a great attention, as the separation of indoor from outdoor environment turn out to be remarkable with extensive sources of tight-sealed buildings and the accompanied sick building syndrome (SBS).

Indoor environment commonly produce large amount of air pollutants, reach human during inhalation, accidental ingestion and dermal contact, including; particulate matter, formaldehyde and bioaerosols. Furthermore, indoor environment can be considered as more hazardous than outside environment, because enclosed spaces can limit

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or restrict aerosols and let them reach the limit of infectious doses. Biological contamination can also flourish in moist components of the system throughout the building². Some people can suffer from respiratory diseases due to the direct effect of most indoor air pollutants on respiratory and cardiovascular systems. Recently, increase of public awareness on food safety related to and environmental problems caused by slaughtering activities attracts attention of administrative authorities and environmental scientists.

Many studies on microbial contaminants in indoor air have been recorded in different environments³. However, the quality of indoor air in slaughterhouses was rarely investigated. Exposure to organic dusts especially in agricultural environment may cause a variety of lung reactions and diseases, such as chronic bronchitis especially due to poultry and animal slaughterhouses⁴. On the other hand, airborne microorganisms are known to cause various health effects, including infections (e.g., acute viral infection, legionellosis, or tuberculosis), hypersensitivity (allergy; e.g., allergic rhinitis, asthma, or hypersensitivity pneumonitis), toxic reactions (e.g., humidifier fever), irritations (e.g., sore throat), and inflammatory responses (e.g., sinusitis or conjunctivitis). Although infectious effects can be caused only by viable pathogens, the allergic, toxic and inflammatory responses can be caused by both viable and non-viable components of the bioaerosol^{1, 3, 5}.

Recently, great concern should be given to the role of bioaerosols in food contamination. Food pathogens namely; *Listeria monocytogenes*, *Bacillus cereus*, and especially *Staphylococcus aureus* are often associated with meat products⁶. *Staphylococcus aureus* is often used as an indicator of food-handler hygiene, because it is found extensively in slaughtering environments^{7, 8}. Environmental pathogens such as *Pseudomonas aeruginosa* also occur generously in food processing environments, because they are able to survive at low temperatures^{9, 10, 11}. It is reported that, members of enterobacteriaceae family, molds, yeasts and staphylococci are increased after cutting, and washing of the carcasses. Moreover, contaminated meat also presents a health risk due to production of mycotoxins. The main sources of moulds contaminating carcasses are air, water, walls

and floors of slaughterhouses¹². Many scientists^{12, 13} reported that *Aspergillus*, *Penicillium*, *Cladosporium* and *Mucor* were frequently isolated from the floors and walls of slaughterhouses.

Although considerable attention has been given to microbial contaminants associated with processing of meat in poultry houses^{14, 15, 16}, limited investigations have reported on the indoor air quality^{17, 18}. Whyte *et al*¹⁹ reported that poultry processing plants namely; working surfaces, equipment, and the hands of workers are susceptible to indoor air contamination. Moreover, air plays significant role in the transmission of pathogens and may be implicated in contamination of poultry meat at various stages of slaughtering and processing^{9, 18}. Fecal contamination of beef carcasses with *Escherichia coli* and *Salmonella* sp. sporadically causes problems at slaughterhouses²⁰, however, little is known about the meat slaughterhouses in some cities like Dammam, Saudi Arabia.

The main aim of the study is to evaluate indoor air quality and bioaerosols levels in slaughterhouse in Dammam, Saudi Arabia. Indoor air quality assessment was carried out through estimation of the concentration of some known indicators of air pollution. Microbiological assessment of air quality as well as some slaughter utensils was closely investigated. Emphasis was given to profiling of the isolated bacteria for various heavy metals as well as antibiotic resistance, their distribution and potential hazardous to human health. Possible use of some common disinfectants was also recommended.

MATERIALS AND METHODS

Sampling, enrichment and isolation

Collection of bioaerosol samples from the two laboratories within the slaughterhouse in Dammam, Eastern province, Saudi Arabia, was carried out by the use of STAPLEX microbial air sampler. The apparatus was placed at different sites within the Labs for 5 and 10 min. Furthermore two different types of media were used; for enrichment and isolation of bacteria nutrient agar (NA) medium (Oxoid) with the following composition was used (g/L); Peptone 5; beef 3; NaCl 5 and agar 15. While, for isolation of fungi ready prepared Sabouraud destrose agar medium was used (Oxoid). At the

end of exposure time, plates were incubated at 37°C and 25°C, for bacteria and fungi respectively. Separate colonies were subcultured on solid media and purified colonies were used in further investigations.

For assessment of other tools and equipments, sterile cotton swabs were used. For testing, swab samples taken from surfaces such as knives, sinks, floors in addition to hands. Swabs were streaked on the surface of agar plates as well as enriched on universal tubes containing nutrient broth medium. At the end of 2 days incubation period, sample was taken from each universal tube, plated on NA medium for 24 to 48 h. Resulted colonies were purified and stored in refrigerator to be used for further analysis.

Water analysis

Water samples were taken from sinks under aseptic conditions and the number of *E coli*/coliform bacteria was estimated by the use of MPN method, by initial cultivation on lactose lauryl tryptose broth medium in the presumptive test²¹. Positive results were confirmed through cultivation on BGGB medium, finally completed test was carried out by transfer to LLB and gram stain.

Antibiotics and heavy metals profiling

Purified bacterial strains were assessed for antibiotic resistance through sensitivity test using Mueller Hinton agar medium as well as Agar Sensitivity Test medium. Eight antibiotics were used in this study namely; Ampicillin (10 µg), tetracyclin (30 µg), erythromycin (5 µg), Streptomycin (10 µg), Kanamycin (30 µg), Ciprofloxacin (5 µg), Amoxicillin (30 µg) and Neomycin (30 µg). All antibiotics purchased from (BD BBL™, USA), except Amoxicillin from (HARDY diagnostic, USA). For heavy metal resistance profiling, different concentrations of the following metals were tested; AgNO₃, ZnSO₄, Cd Cl₂, PbNO₃ (stock solution: 4 mg/ml); K₂Cr₂O₇ (stock solution: 2 mg/ml) and HgCl₂ (stock solution: 1 mg/ml).

Cultivation on other selective and chromogenic media

Other media used in this study include; MacConkey agar media (Bioworld, USA) with bile salt mixture³ (Bioworld, USA) for enteric bacteria, Mannitol salt agar medium (Bioworld, USA) for Staphylococci and Streptococci and Blood agar medium for hemolytic bacteria (Bioworld, USA).

Table 1. Isolation of bacteria and fungi from air and other utensils in Dammam slaughterhouse.

Source	Bacteria Description	Fungi Description
Lab. A: Air (5 min) Air (10 min)	<i>Streptococcus</i> sp. strain-AS5/1, <i>Staphylococcus</i> sp. strain-AS5/2 <i>Bacillus</i> sp. strain-AS 10/1, <i>Mycobacterium</i> sp. strain-AS10/3, <i>Staphylococcus</i> sp. strain-AS10/5 and 6, <i>Streptococcus</i> sp. strain-AS10/7	<i>Aspergillus</i> sp., <i>Microsporium</i> sp.
Saw	<i>Staphylococcus</i> sp. strain-S1, and -2, <i>Streptococcus</i> sp. strain-S3	<i>Aspergillus</i> sp., <i>Cryptococcus</i> sp.
Blade	<i>Pseudomonas</i> sp. strain-B1	<i>Cryptococcus</i> sp., <i>Candida</i> sp., <i>Aspergillus</i> sp., <i>Penicillium</i> sp., <i>Rhodotorula mucilaginosa</i>
Knife	<i>Staphylococcus</i> sp. strain-KA1	<i>Cryptococcus</i> sp., <i>Candida</i> sp., <i>Trichophyton</i> sp.
Lab. B: Air (AS2) (10 min)	<i>Bacillus</i> sp. strain-AS2/11, <i>Staphylococcus</i> sp. strain-AS2/12, Coliform bacteria-AS2/21, <i>Micrococcus</i> sp strain-AS2/22. - <i>Salmonella</i> sp. strain-AS2/31, <i>Mycobacterium</i> sp. strain-AS2/32	<i>Cladosporium</i> sp., <i>Candida</i> sp., <i>Microsporium</i> sp.
Knife	<i>Staphylococcus</i> sp. strain-KB/1, <i>Pseudomonas</i> sp. strain-KB/3	<i>Cladosporium</i> sp., <i>Candida</i> sp.

RESULTS

Assessment of Indoor Microbial Contamination in Slaughterhouse

In this experiment, bioaerosol level in indoor air of two laboratories, Dammam slaughterhouse, Saudi Arabia, was closely examined by enrichment and isolation of bacteria and fungi on nutrient agar as well as Sabouraud dextrose agar media, respectively. To test the effect of other microbial communities in background that might affect bioaerosols and hence air quality, microorganism from other utensils were also enriched and isolated. Preliminary studies indicated that; several bacterial strains can be isolated from air, water, sinks, saws, blades and knives by cultivation on nutrient agar media. Moreover, few fungal strains were isolated from the same sources by cultivation on Sabouraud dextrose agar media. Results in **Table 1** refer to summarized description of the most dominant bacterial and fungal candidates enriched and isolated, from the two Labs. The most common bacteria were; *Salmonella* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., and *Bacillus* sp., while fungi include; *Aspergillus* sp., *Microsporium* sp., *Trichophyton* sp., *Rhodotorula mucilaginosa*, *Candia* sp., and *Cryptococcus* sp.

Interestingly, total bacterial count, after 5 and 10 min work of Staplex microbial air sampler in Laboratory A and B was 77 and 43 CFU/m³, respectively. While, constant bacterial count was recorded after 5 and 10 min working near directors offices with a value of 25 CFU/m³. On the other hand, enumeration of total coliforms in washing water of the two laboratories, by MPN method, recorded higher concentration of bacteria with a value of 39 CFU/100mL.

Profiling of Antibiotics and Heavy Metals Resistance

To get more information about the possible hazards encountered by bacteria, representative candidates were assessed regarding antibiotic as well as heavy metals resistance. In the first experiment, eight different antibiotics were used to profile antibiotic resistance among bacterial bioaerosols as well as some bacteria enriched during background studies. Results graphically presented in **Figure 1** indicated that bacterial strains *Streptococcus* sp. strain-AS10/7, *Staphylococcus* sp. strain-KA/1 and *Staphylococcus* sp. strain-KB/1 with highest resistance to streptomycin. The strain *Staphylococcus* sp. strain-AS5/1 showed highest resistance to ampicillin and erythromycin. Interestingly, bacterial strains isolated from air of

Table 2. Growth of some bacterial candidates on blood agar, MacConkey as well as mannitol salt agar chromogenic media

Organism	Blood Agar	MacConkey Agar	Mannitol salt agar (MSA)
<i>Staphylococcus</i> sp. strain-S1	Non	Red*	-
<i>Staphylococcus</i> sp. strain-S3	Non	-	-
<i>Pseudomonas</i> sp. strain-B1	Non	-	-
<i>Staphylococcus</i> sp. strain-KA/1	Non	Red*	Yellow
<i>Streptococcus</i> sp. strain-AS5/1	α-hemolytic	-	-
<i>Staphylococcus</i> sp. strain-AS5/2	β-hemolytic	-	Yellow
<i>Staphylococcus</i> sp. strain-S10/5	Non-hemolytic	-	-
<i>Streptococcus</i> sp. strain-AS10/7	α-hemolytic	-	Yellow
<i>Staphylococcus</i> sp. strain-KB/1	Non	-	-
<i>Bacillus</i> sp. strain-AS2/11	Non	-	-
<i>Staphylococcus</i> sp. strain-AS2/12	Non	-	-
<i>Salmonella</i> sp. strain-AS2/21	Non	Red	-
<i>Micrococcus</i> sp. strain-AS2/22	Non	Red	-
<i>Mycobacterium</i> sp. strain-AS2/32	β-hemolytic	-	-

Note: * Pale color, no growth or no color change

the Laboratory B (except; AS2/11) showed the highest sensitivity towards all tested antibiotic, followed by bacterial strain *Staphylococcus* sp. strain-AS5/2. However, bacterial strains *Streptococcus* sp. strain-AS5/1 (Lab. A) and *Streptococcus* sp. strain-AS10/7 (Lab. B) showed moderate resistance to amoxicillin.

On the other hand, profiling of heavy metal resistance indicated higher concentrations of heavy metals led to more toxic effect, reflected by the increase in inhibition zone (Figure 2,3). Interestingly, all bacterial candidates, from air of Lab. B were highly sensitive to all tested heavy metals. Most of the strains showed moderate resistance to silver ions. While, lower concentration of mercury was highly toxic to all tested bacteria, chromium ions developed more toxicity when used at higher concentrations. Interestingly, all bacteria isolated from air as well as other utensils showed less sensitivity to 10% Clorox solution at lower

concentration. However, higher concentrations of 10% Dettol solution showed the highest effect with nearly all tested strains except the most resistant candidate *Staphylococcus* sp. strain-AS5/2.

Biochemical activities

For further biochemical characterization several chromogenic and diagnostic media were used. In this experiment each bacterial candidate was subcultured on Blood agar, MacConkey and Mannitol salt agar media. Results shown in Table 2 indicated that *Staphylococcus* sp. strain-AS5/2 and *Mycobacterium* sp. strain-AS2/32 showed Beta-hemolysis activity, while *Streptococcus* sp. strain-AS5/1 and strain-AS10/7 showed alpha-hemolytic activity, which is a serious indication for possible hazards and toxicity of those isolates. Furthermore, other bacterial strain e.g. *Pseudomonas* sp. strain-B, *Bacillus* sp. strain-AS2/11, other *Staphylococcus* sp. strains and *Micrococcus* sp. strain-AS2/22 showed no

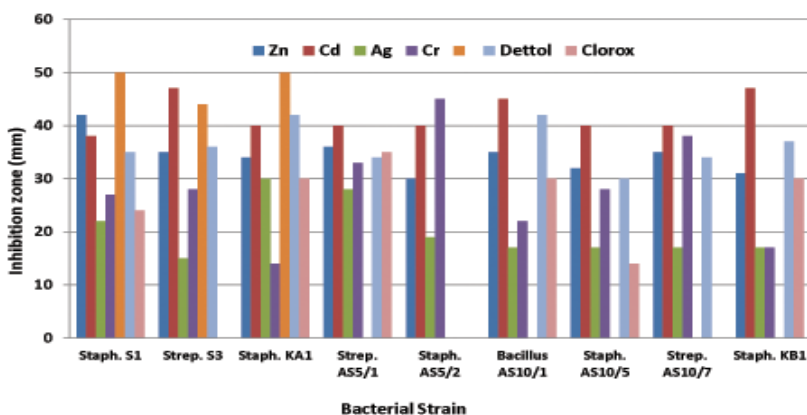


Fig. 1. Antibiotic profiling of bacterial candidates isolated from Dammam Slughterhouse

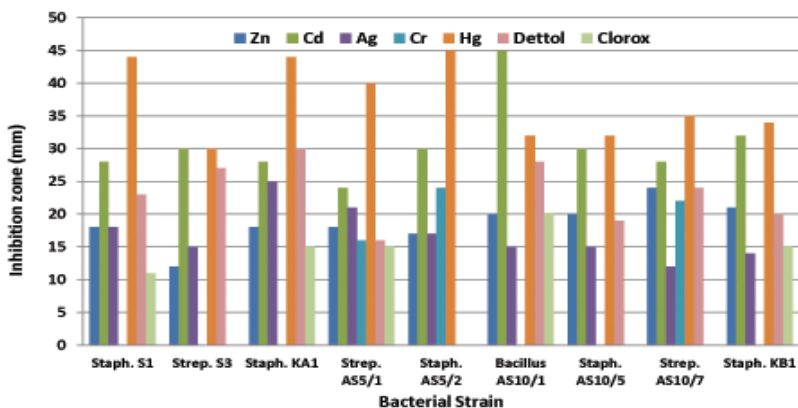


Fig. 2. Heavy metals profiling (lower conc.) of bacterial candidates isolated from Dammam Slughterhouse

hemolytic activity, thus could be harmless in case of accidental human contact.

On the other hand, most of bacterial strains showed no or slight growth on MacConky agar medium with pale color change except *Salmonella* sp. strain-AS2/21 that was able to ferment lactose and produce red color. Furthermore, bacterial strain *Staphylococcus* sp. strain-KA/1, *Staphylococcus* sp. strain-AS5/2 and *Streptococcus* sp. strain-AS10/7 showed clear growth on mannitol salts agar medium (MSA) with clear yellow color due to utilization of mannitol.

DISCUSSION

In a program to assess microbiological as well as bioaerosol quality in Dammam slaughterhouse, microbial candidates that might exist in air and other utensils within two laboratories were closely examined. It was clear that several bacterial candidates can be isolated from air, saw, blades and knives by cultivation on nutrient agar media. Moreover, few fungal strains were isolated from the same sources by cultivation on Sabouraud dextrose agar media. The most common bacteria were; *Salmonella* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Streptococcus* sp., *Micrococcus* sp., and *Bacillus* sp., while fungi include; *Aspergillus* sp., *Microsporium* sp., *Rhodotorula mucilaginosa*, *Candia* sp., and *Cryptococcus* sp. It is essential to keep concern about the airborne bacteria in slaughterhouses because it is clearly have impact on the meat. This is supported by the finding of other working group²², they found that airborne

bacteria have significant role in carcass contamination. Remarkably, bacterial candidates belong to genus *Staphylococcus* sp. were most common in slaughterhouse bioaerosol and other utensils in Dammam. In concordance with this finding, several scientists often used *Staphylococcus* sp. candidates as an indicator of food-handler hygiene, because it is found extensively in slaughtering environments^{7, 8}. Moreover, large number of bacteria were isolated on specific chromogenic media namely; *E. coli*, *Salmonella*, *B. cereus*, *S. aureus* and the environmental pathogen *Pseudomonas aeruginosa* from bioaerosols of a high-throughput chicken-slaughtering facility in South Africa²³. Furthermore, Assessment of air safety in a German Turkey house was investigated and the airborne bacteria were detected by cultivation-based as well as molecular methods, especially for species with a potential health risk for employees (*Acinetobacter johnsonii*, *Aerococcus viridans*, *Pantoea agglomerans*, and *Shigella flexneri*)²⁴. In another study six genera belong to *Penicillium* sp. and *Aspergillus* sp. were detected in air, water and equipments in two slaughterhouses in Serbia²⁵. In this study, the pathogenic fungus sp. *Cryptococcus* sp. and non-pathogenic *Rhodotorula mucilaginosa* were detected. Indeed, fungal candidates belong to dermatophytes was also detected which gives an indication for the high risk encountered by those bacteria and fungi. Owing to higher concentrations of microbial candidates in the air of the two laboratories, it is recommended to use special HEPA filters to avoid the possible transmission of airborne pathogens

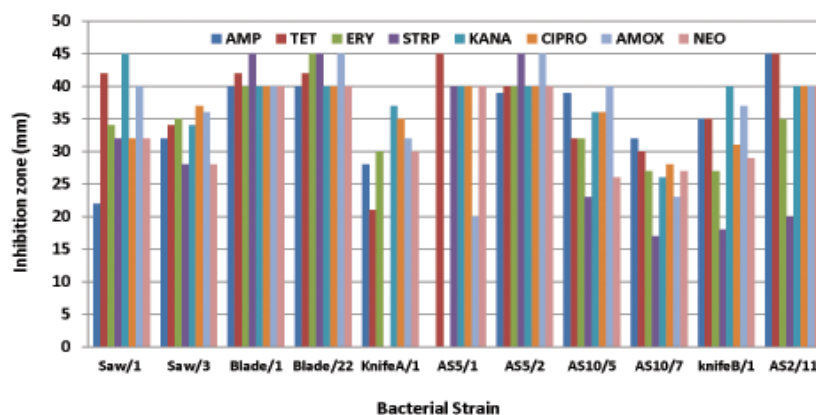


Fig. 3. Heavy metals profiling (higher conc.) of bacterial candidates isolated from Dammam Slaughterhouse

that could exist. Unfortunately, washing water in both laboratories exceeds the international standards for purified water, hence not recommended to be used in slaughterhouses²¹. Therefore, use purified water as well as regular sanitization of water tanks is recommended. Interestingly, bacterial candidates isolated from air showed higher sensitivity to antibiotics and heavy metals, in comparison with the background bacterial isolates. However, some candidates showed multiple tolerances to both heavy metals and antibiotics. Horizontal gene transfer among background bacteria occur in many utensils might play an important role in transfer of antibiotic and heavy metal resistance. It is thought that a correlation exists between metal tolerance and antibiotic resistance in bacteria²⁶ because of the likelihood that resistance genes are encoded on the same plasmid in bacteria^{27,28}.

Besides, the *Staphylococcus* sp. strain-AS5/2 and *Mycobacterium* sp. strain-AS2/32 showed Beta-hemolysis activity, while *Streptococcus* sp. strain-AS5/1 and *Streptococcus* sp. strain-AS10/7 showed alpha-hemolytic activity, which is a serious indication for possible hazards and toxicity of those isolates. It also reflects the possible risk encountered by those isolates in case of direct contact with human. Unfortunately, most of bacterial candidates were less sensitive to 10% Clorox, however, 10% Dettol showed a high bactericidal activity. Therefore, it is recommended to apply 10% Dettol to achieve optimal hygienic standards and to keep employees and consumers safe and healthy.

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