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Systematic Review and Meta-Analysis on *Staphylococcus aureus* in Ethiopia

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ABSTRACT

Staphylococcus aureus was the first bacteria against which drug/penicillin/ was discovered and what has become that has been challenging for its drugresistant property throughout the world. Monitoring the status of S. aureus both its susceptible and drug-resistant properties is pretty important to develop optional preventive methods timely. Even though fragmented studies on the apparent prevalence of S. aureus in milk and meat have been conducted in Ethiopia, a well-organized national review on this pathogen remained scarce. A systematic review and meta-analysis of S. aureus was conducted to overview the pooled apparent prevalence in Ethiopia in a ten-year period from 2011 to 2020. PubMed and Google Scholar databases were used to search for research articles published in reputable journals. Lastly, twenty-nine research articles were selected for systematic review and meta-analysis. The forest plot pooled the apparent prevalence of S. aureus with a 95% CI. The overall pooled apparent prevalence of any detection using the random effect model was 23% (95% CI: 19–28%, p<0.001). The pooled apparent prevalence of S. aureus was highest in SNNP 39% (95% CI; 15– 63%), followed by Oromia 28% (95% CI; 18–38%). The minimal effect size was observed in Amhara regional states at 5% (95% CI; 3–7%). This review indicated that the apparent prevalence of S. aureus more or less decreased from 27.8% in 2012 to 11.4% in 2020. In conclusion, high variation and decreasing trend of the apparent prevalence across different regions was observed.

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Introduction

In the recent years, the earth is facing diverse challenges from which, climate change and massive population growth was found among others. As the climate is changing from time to time, micro-organisms are also changing themselves to adapt and fit the environment for their survival, leaving the common preventive methods or drugs in to no help or at least minimizing its previous effective importance. The first drug discovery breakthrough was in the 1940s by Alexander Fleming and from that time onwards, different antibiotics have been used to treat humans effectively [1]. However, through time, the efficacy of commonly used drugs has been decreasing due to the creation of drug resistance, even some bacteria become multi-drug resistant [2].

Staphylococcus aureus is among the common bacterial pathogens that emerge as the health problem to human and their animals [3]. As indicated in review article by Kumar and his colleagues [4], the methicillin-resistant *S. aureus* (MRSA) was the first ringing bell as a breakthrough for the drug-resistant bacteria. On the other hand, the drug-susceptible *S. aureus* has also been creating health threatening to human and their livestock in places where the presence of antibiotics was scares.

Most developed countries like America, Europe, and Australia conduct frequent researches on the drug-resistant and drug-resistant *S. aureus all the time [4]. As a result, the proportion of MRSA surpasses the proportion of MSSA from 20 to 80% demanding alternative drugs to treat infections caused by this agent. In developing countries, the proportion of MRSA to MSSA could be even beyond the studies reported in the developed countries due to the in appropriate use of drugs both in the medical and veterinary uses. In spite of certain improvements in recent years, in rural areas of African countries, including Ethiopia, veterinary drugs can be sold in open markets as any shopping goods, which leads to a wide distribution of drug-resistant bacteria. Meta-analysis on methicillin-resistant S. aureus from 2004 to 2015 in Ethiopia indicated higher prevalence of the causative agent [5]. However, the apparent prevalence of S. aureus (both susceptible and drug-resistant) in the last ten years in the country is not well analyzed and documented. Therefore, the objective of this systematic review and meta-analysis was to summarize available data, determine the pooled apparent prevalence of S. aureus in the last ten years (2011-2020) in Ethiopia*.

Methods Study Selection

PubMed and Google Scholar databases were used for literature search of articles, and articles potentially relevant to the study were selected. The search mechanism was conducted by using the following terms as keywords (and combinations thereof)

"*Staphylococcus aureus*", "S. aureus", "prevalence" and "Ethiopia". From the extracted citations, first abstracts were reviewed to retrieve the apparent prevalence studies on S. aureus. Relevant original articles, by title and abstract, were further observed in full text to determine those that provided enough information to be included in the systematic review and meta-analysis. At last, the references cited by each relevant study were inspected to identify additional articles.

Inclusion and Exclusion Criteria

Original articles were included in the meta-analysis, if they reported extractable data on the apparent prevalence of *S. aureus* in Ethiopian cattle meat and milk, and camel milk. Articles written only in English language were included in the meta-analysis. Studies that did not report on a study of *S. aureus* and failed to comply with the Ethiopian situation were omitted from the analysis. Articles published only in reputable journals were considered in this meta-analysis. Selection of reputable journals was conducted using the article published by Elmore and Weston which states "Predatory Journals: What they are and how to avoid them"[6]. Accordingly, the like of 'The Directory of Open Access Journals (DOAJ)', 'The Committee on Publication Ethics (COPE)', 'SCImago Journal Rank', 'National Library of Medicine (NLM) Catalog' and 'Stop Predatory Journals' were employed to check whether the article was relevant or not to be included in meta-analysis.

Outcome of Interest

The main center of attention was the apparent prevalence of *S. aureus* isolated from cattle meat, milk, camel milk and their products. The apparent prevalence was calculated by dividing the numbers of samples that have *S. aureus* isolates to the total number of samples taken from cattle meat, milk and camel milk, and their products.

Data Extraction and Quality Control

Data obtained from relevant studies were mined and summarized into an excel spreadsheet. From every involved study, name of regions, study area/city, study names, year of the study, study design, types of specimens, numbers of study participants, total numbers of S. aureus, and references were included. The quality of relevant data/studies was tested using a set of predetermined criteria such as research design, quality of paper and employed methods for *S. aureus* isolation.

Data Analysis

Data extracted from articles were prepared in Excel spreadsheet and inspected and ready for analysis. Data of each study area was set to have presence or absence with its weight as an outcome/dependent variable, and study areas (regions), years and source of samples were considered as independent variables. A random effects model was used to determine the pooled apparent prevalence and the 95% confidence interval (CI), by employing the approach of DerSimonian and Laird [7]. According to Huedo-Medina et al. [8], random effect is used over the fixed effect model to minimize the heterogeneity effect of the data.

The standard approach of inverse variance method to calculate pooled apparent prevalence does not work well in meta-analysis of single arm study because, for studies with small or large apparent prevalence, the inverse variance method causes the variance becomes small and the calculated CI may become outside the range. Therefore, Freeman Tukey arcsine methodology is recommended to correct both variances instability and CIs [9]. The heterogeneity of the study results was assessed using I2 test. Significant heterogeneity was considered for $p< 0.10$ and $I2 > 50\%$ [7], [10]. The overall apparent prevalence of *S. aureus* was pooled by forest plot with 95% CI, and the regional apparent prevalence was summarized by using figure. Stata16 software was used for all statistical analysis.

Results

A total of 114 articles (71 from Pub Med and 43 from Google scholar) were retrieved using the electronic database search. Of the 114 articles, 93 of them were excluded due to the inclusion/exclusion criteria mentioned in the methodology. Four articles were also excluded due to duplication in PubMed and Google scholar remaining 14 from PubMed and 3 from Google Scholar. Additionally, 12 articles/ data were mined from the original existing articles that fulfill the inclusion criteria mentioned in the methodology. Finally, 29 articles were considered as relevant to be included in the systematic review and meta-analysis from which, 4837 samples were retrieved (Table 1).

Characteristics of Included Studies

All the study articles and extracted data included here were laboratory-based cross-sectional studies. Based on the source of the sample type, twenty six articles/ extracted data were from milk samples, of which, one article was from camel milk. Three of the studies were on beef obtained from abattoir (Table 1).

Meta-Analysis

The Heterogeneity and publication bias was first examined. Accordingly, the analysis showed substantial heterogeneity among the studies (Cochran's Q test = 599.92, $p<0.001$; $I2 = 95.33.0\%$, $p<0.001$) (Figure 1).

Pooled Apparent Prevalence of *Staphylococcus aureus*

The pooled apparent prevalence of *S. aureus* was determined from the 29 studies. The overall pooled apparent prevalence of any detection using the random effect model was 23% (95% CI: 19–28%, $p<0.001$). The highest effect size was observed to be 51% (95% CI: 44-59%) by Abebe eat al. (2016) in Hawassa milk shade. On the other hand, the lowest was found to be 5% (95% CI: 3-7%) by Bihon et al. (2018) in Gondor (Figure 1 and Table 1).

Table 1: Summary of *Staphylococcus aureus* apparent prevalence studies in different parts of Ethiopia, 2011-2020

Author and year	Region	District	Sample used	Detection method	S. size	A. prevelence
[11]	Tigray	Mekelle	Cow milk	Molecular	385	12.5
[12]	Tigray	Mekelle, Wuk. , Adig.	Cow milk	Bacterial culture	315	11.4
[13]	Tigray	Shire	Cow milk	Bacterial culture	220	9.5
[14]	Oromia	Yabello	Cow milk	Bacterial culture	126	11.1
[14]	Oromia	Yabello	Camel milk	Bacterial culture	44	11.4
[15]	Oromia	Mukaturi	Cow milk	Bacterial culture	78	21.8
[15]	Oromia	Sululta	Cow milk	Bacterial culture	105	10.5
[16]	Amhara	Gondor	Cow milk	Bacterial culture	334	5.1
[17]	A. Ababa	Addis Ababa	Beef from abattoir	Bacterial culture	384	9.4
[17]	A. Ababa	Addis Ababa	Beef from butcher	Bacterial culture	384	19.8
[18]	Oromia	Bishoftu	Subclinic. mastitic cow	Bacterial culture	262	45.0
[19]	A. Ababa	Addis Ababa	Cow pooled milk	B. culture & bioch.	48	20.8
[19]	A. Ababa	Addis Ababa	Meat from abattoir	B. culture & bioch.	103	11.7
[20]	Oromia	Sebeta	Cow milk	B. culture & bioch.	209	19.6
[21]	Oromia	Asella town	Mastitic cow milk	Bacterial culture	230	46.5
[22]	Oromia	Jimma	Cow milk from cows	B. culture & bioch.	32	37.5
[22]	Oromia	Jimma	Retail&colln. center's cow milk	B. culture & bioch.	42	35.7
[23]	Tigray	Mekelle	Cow milk & milk products	B. culture & bioch.	147	31.3
[23]	Tigray	Shireendasilasie	Cow milk &milk products	B. culture & bioch.	139	43.2
[23]	Tigray	Addigudem	Cow milk &milk products	B. culture & bioch.	52	30.8
[23]	Tigray	Adigrat	Cow milk &milk products	B. culture & bioch.	43	20.9
[23]	Tigray	Wukro	Cow milk &milk products	B. culture & bioch.	60	25.0
[23]	Tigray	Abi-Adi	Cow milk &milk products	B. culture & bioch.	34	8.8
[23]	Tigray	Maichew	Cow milk &milk products	B. culture & bioch.	53	15.1
[24]	SNNPR	Awasa milk shade	Cow milk	B. culture & bioch.	172	51.2
[25]	Oromia	Bishoftu	Bulk cow milk	B. culture & bioch.	170	43.5
[26]	SNNPR	Awasa	Cow milk	B. culture & bioch.	160	48.8
[27]	SNNPR	Awasa	Cow milk	B. culture & bioch.	201	16.9
[28]	Tigray	Mekelle	Cow milk	B. culture & bioch.	305	15.1

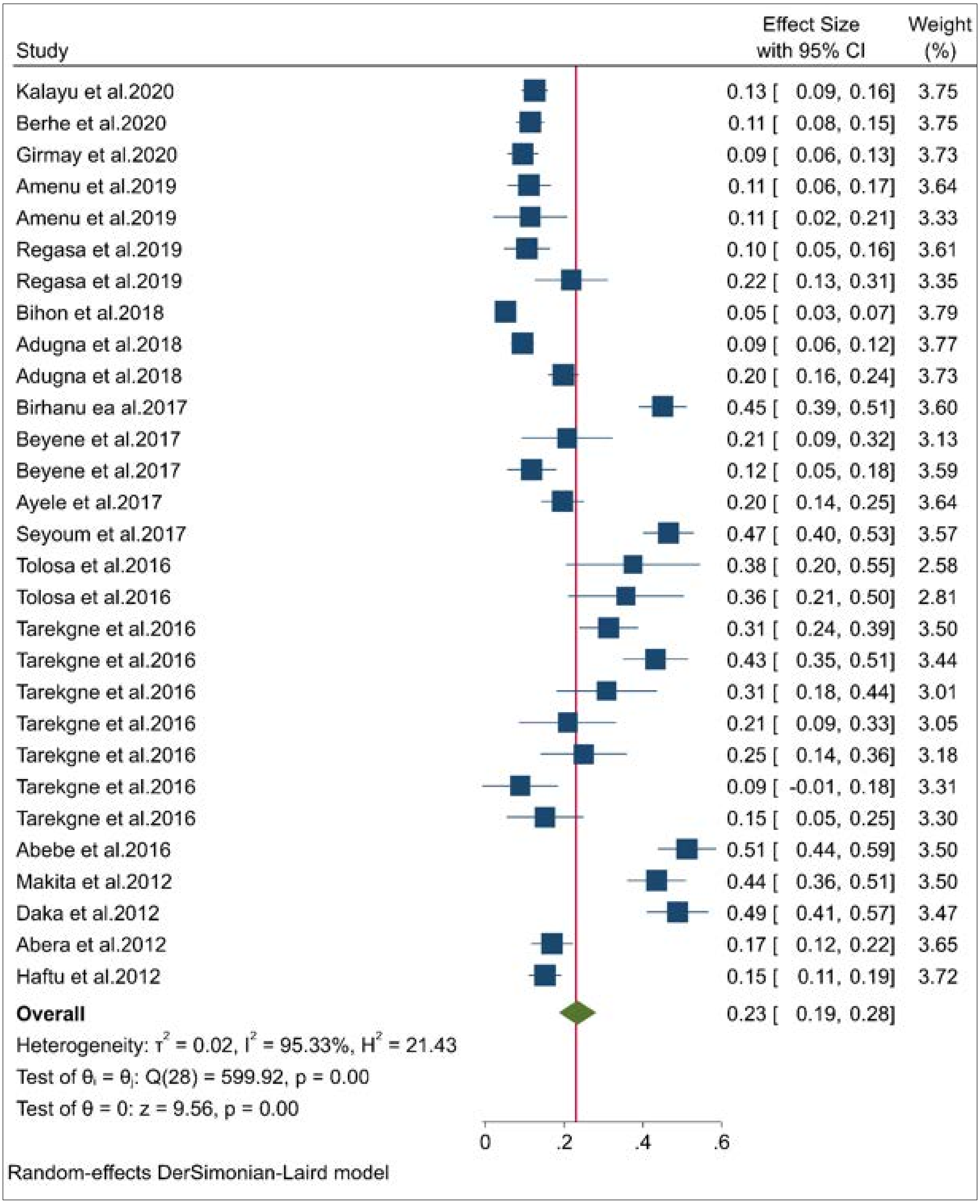

Figure 1: Forest plots indicating the pooled proportion of *S. aureus* in Ethiopia in ten year time period (2011-2020)

Subgroup Analysis

Subgroup analysis is pretty important in decreasing the heterogeneity of the studies in different locations. Consequently, subgroup analyses were conducted on the nine regional states and two administrative cities. The pooled apparent prevalence of *S. aureus* was highest in SNNP 39% (95% CI; 15– 63%), followed by Oromia 28% (95% CI; 18–38%). The minimal pooled apparent prevalence was observed in Amhara regional states 5% (95% CI; 3–7%) (Figure 2). On the other hand, effect size for the pooled apparent prevalence was not calculated for Gambella, Afar, Somali, Benishangul-Gumuz and Harari regional states, and Dire Dawa administrative City as there was no data that fulfill the inclusion criteria of this review method.

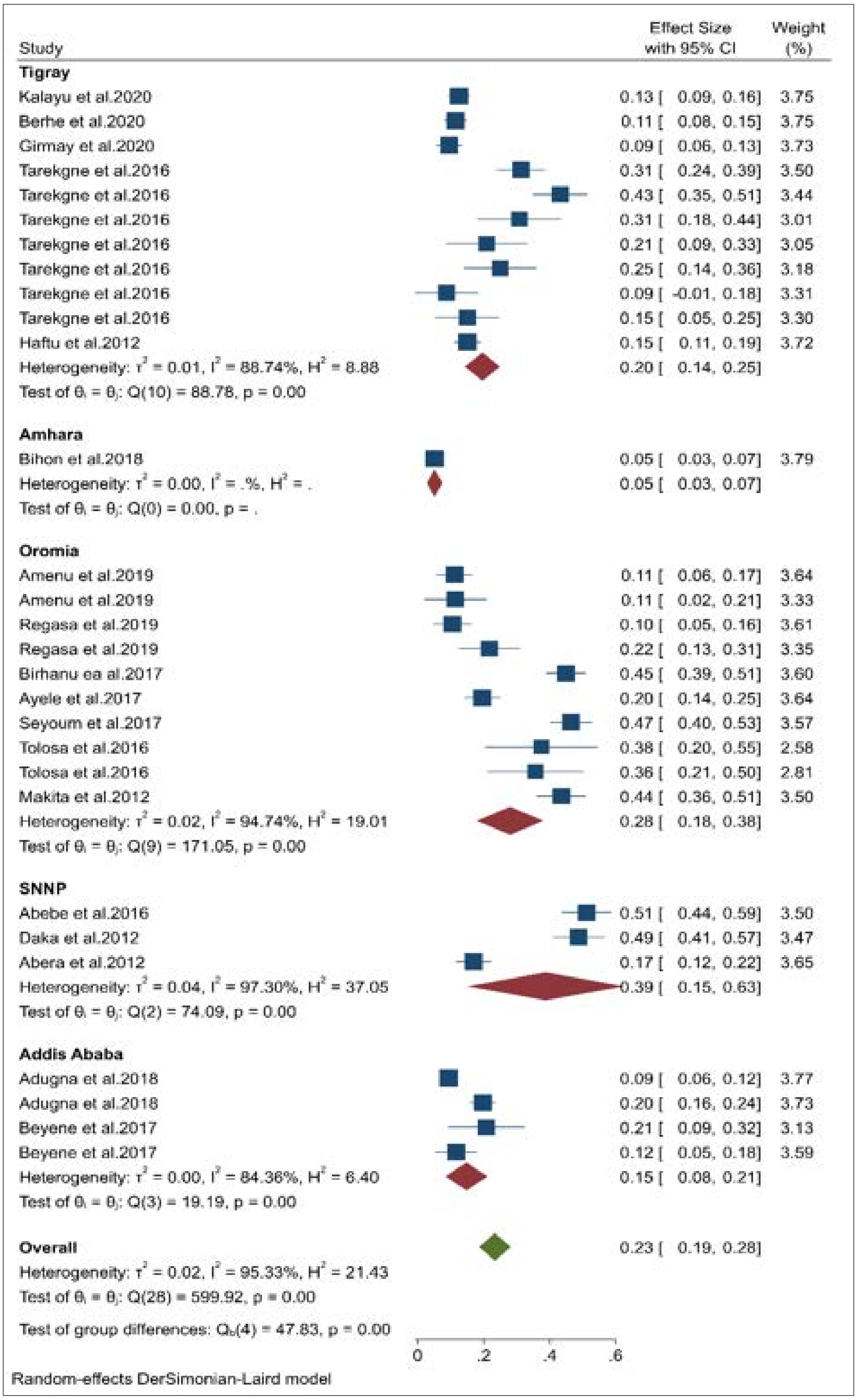

Figure 2: Forest plots: Proportions of *S. aureus* by region

As indicated in Figure 3, the cumulative effect size of the pooled apparent prevalence of *S. aureus* in the 10 year-time period had been decreased from 44% in 2012 to 23% in 2020. Except from the years of 2012 to the years of 2016, the cumulative effect size had been decreasing in almost a linear way.

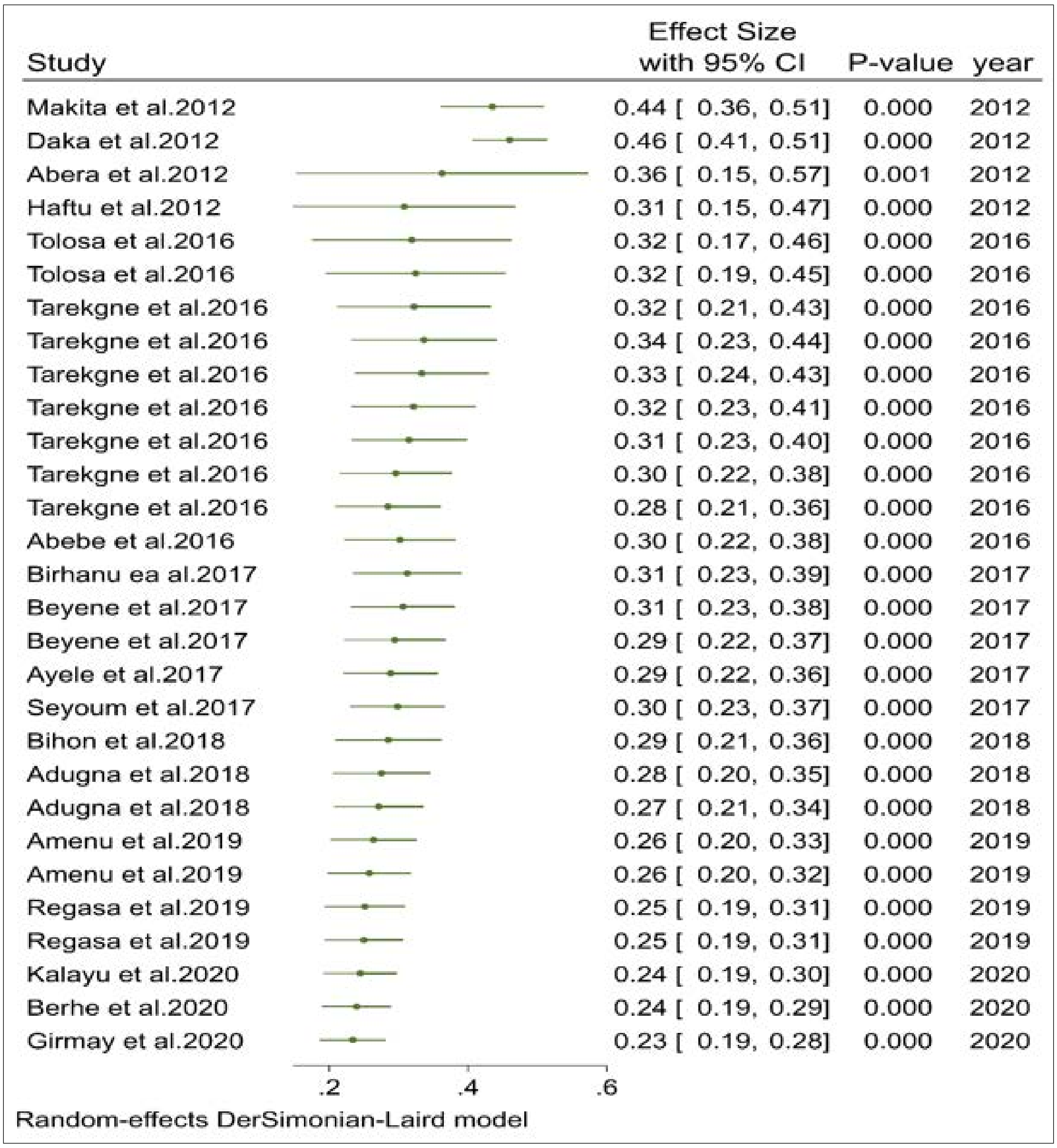

Figure 3: Cumulative analysis of the pooled proportion of *S. aureus* from 2011-2020

Discussion

Staphylococcus aureus is among the agents that cause dairy cows teat blindness and mastitis [29]. The fast drug resistant properties of *S. aureus* makes it one of the public health concern across the world [30]. Being one of the six pathogens associated to death due to its drug resistance in 2019 attracts the attention of the world [31]. In Ethiopia, certain number of systematic review and meta-analysis on the drug resistance of *S. aureus* has been documented. However, to the best level of my understanding, this is the first systematic review and meta-analysis of *S. aureus* in cow meat and milk, and camel milk products in both the drug resistant and drug susceptible in Ethiopia.

The overall pooled apparent of *S. aureus* from milk and meat in Ethiopia in the period of 2011 to 2020 was found to be 23% (95% CI: 19-28%). The pooled apparent prevalence of *S. aureus* in our study was more or less similar with a finding of 25% (95% CI: 21-29) from 137 number of studies in milk [32]. Another study from Ethiopia revealed pooled estimate of Staphylococcus spp. isolated from meat samples at 21% (95% CI: 12-30) from 27 relevant articles [33]. On the other hand, higher pooled apparent prevalence of *S. aureus* contamination in beef was reported (29.7%, 95% CI, 18.4 - 42.3%) from a world-wide review [34]. Comparing to this study, higher pooled apparent prevalence of *S. aureus* (35.8%, 95% CI: 30.4- 41.5%) was reported in seven African and Asian countries from ready to eat foods [35]. These variations in the pooled apparent prevalence could be due to variation level of understanding the risk and contamination mechanism of *S. aureus* [20]. Dairy and meat activities managed by workers who have the awareness of *S. aureus* can be lowered the risk of contamination to the products than those without.

One interesting finding of our review is that the pooled apparent prevalence of *S. aureus* in Ethiopia was decreased from 2011 to 2020 (Figure 3). Similar finding was observed in chicken meat contamination in that, the pooled apparent prevalence of *S. aureus* was decreased from 46% in 2000 - 2011 to 24% by the years 2011 - 2016 [34]. The possible reason for this scenario is that, while the drug susceptible *S. aureus* decreases, drug-resistant once increases from time to time. In spite of the fact that the overall apparent prevalence of *S. aureus* (drug resistant and drug susceptible) seems decreasing, the drug resistant strains have been increasing. This implies that the drug resistant strain population is taking over the drug susceptible strain population. A drug resistance systematic review and meta-analysis conducted world-wide from 1969 to 2020 revealed that the prevalence of drug resistance was increasing from time to time, more apparently from 2009 onwards [36]. Hundreds of thousands of death has been rising each year [37]. This will be the most dangerous situation in the near future especially in the developing countries in that, over 10 million yearly deaths will be triggered by antimicrobial-resistant pathogens by the year 2050 [38].

According to the review methods we followed, six out of the nine regions and two administrative cities were included in this study. Out of the 29 articles, more than 50% were from Oromia and Addis Ababa. The pooled apparent prevalence of *S. aureus* showed statistically significant difference (Q= 47.83, p <0.001) among the administrative regions. The highest was found in SNNP (39%, 95% CI: 15-63) followed by Oromia (28%, 95% CI: 18-38%). Whereas the list apparent prevalence was found in Amhara regional state (5%, 95% CI: 3-7%). Meta-analysis conducted on nasal carriage of human being in Ethiopia indicated that higher variation among regions was observed [39]. Sub-group meta-analysis of *S. aureus* pooled apparent prevalence was also observed in a statistically significant variation in different countries (I2= 94.93, p<0.001) [35]. Possible reason for this variation could be miss-use of antibiotics in farm animals [40]. Farmers/ dairy and fattening owners who have the easy access to antibiotics in the market frequently used to treat their animals without the consent of professionals. This leads to high number of drug resistant bacterial population.

Conclusion

In conclusion, higher apparent prevalence of *S. aureus* was observed across different study regions of the country. The cumulative analysis indicated that *S. aureus* was decreasing from time to time within the study time period. However, the author would like to stress here is that this review article did not separate the drug-resistant and drug-susceptible strains of S. aureus.

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Disclosure

The author would like to express that there is no conflict of interest in this review article.

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